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STUDIES ON SPORE FORMATION AND SPORE RESISTANCE*

O. B. Williams

Despite the fact that bacterial spores have been the subject of experimental investigation almost from the time they were first recognized there is still no unanimity of opinion either as to their function in nature, or as to the related problem of the factors concerned in their formation. Since no multiplication takes place as a result of the vegetative cell—spore—vegetative cell cycle, few bacteriologists accept the concept of the spore as a cell set apart for reproduction. Instead various explanations of the biological nature and function of bacterial spores have been advanced, including the teleological interpretation of the spore as a resistant structure produced to enable the organism to survive an unfavorable environment, the idea that a spore is a normal resting state—a form of hibernation, and the notion that spores are stages of the developmental cycle of certain organisms, or are a provision for the rearrangement of nuclear material.

Most opinions on the biological nature of spores are compatible with the interpretation of the spore as a reproductive structure. The spores formed by certain algae, and by some protozoa in the course of a modified form of sexual reproduction, for example, are accepted as reproductive in nature. The lack of multiplication—the single cell to spore to single cell cycle—has not been used as an argument against the reproductive nature of these structures, which have a developmental history similar to that of bacterial spores. Not only among lower plants and animals, but also among higher plants a comparable situation may occur. Thus, for example, a single seed may give a plant which forms a corm, or a bulb, which in turn will grow into another single plant. The bulb and the corm are each considered by botanists to be reproductive structures. Furthermore, seeds and other bonafide reproductive

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structures of plants and animals show resistance to environmental conditions and possess a distinct dormancy. Therefore, it would not seem justified to rule out the reproductive nature of spores on these grounds. The microbiologist who would ascribe some special function other than reproduction to the bacterial spore must assume the responsibility of parenthood for a system of terminology and concepts for bacteria which are not in full accord with established ideas and terms associated with other forms of life.

The question of the biological nature of the spore has less practical importance than other aspects of the general field of spore study. Twenty years ago, factors affecting the heat resistance of bacterial spores were studied by me. At the time this work was undertaken it was hoped that the results would assist in an understanding of the mechanism concerned in the differing resistance among spores of different species and strains and even among different spore crops of the same strain of a single species, as well as the difference in resistance between spore and vegetative cell. Until we know more about what makes the spore behave the way it does we can proceed only by empirical methods in those operations where spore control or destruction is of importance. Since it is possible that there is a close relationship between the physiology of spore formation and spore resistance, attempts have been made in our laboratory to investigate this problem.

A review of the early literature on the physiology of spore formation suggested that more attention might be paid to determinations of the percentage of sporulation in terms of the total amount of growth. It is easy to establish that a large number of spores may be only a small fraction of the total number of cells in a medium, while a much smaller number of spores per unit volume of medium may represent essentially complete sporulation. Both the extent of growth and the degree of sporulation should be determined in order to have a full evaluation of the effect of a nutritive or environmental factor on spore formation.

With some organisms, and under certain cultural conditions with others, it is not practicable or possible to determine numbers by dilution plating. Furthermore, many spores are of

low resistance to heat and may be killed by the heat treatment used to kill the vegetative cells in preparation for a spore count by cultural procedure. For these reasons, though appreciating the lack of accuracy, it was decided to use direct microscopic counting of stained smears to establish both the extent of growth and the degree of sporulation. In some media marked autolysis takes place so that many ghost cells and fragments of cells may be observed. These cause difficulty in counting. However, the direct microscopic count will include many intact cells no longer capable of growth and may, therefore, actually give a more nearly correct approximation of the total number than would cultural counting.

In our early work the organism used was an aerobic spore former designated as the Kahn strain of *Bacillus subtilis*. The strain identification of the organism has been lost and consequently we are not certain that we still have the culture. We are fairly certain that this organism was not identical with that now designated as *B. subtilis* in the Bergey Manual. More recent work, especially with heat resistance studies where ready cultural recognition was important, have dealt with the pigmented *Bacillus globigii*. Some work has been done with a culture designated as *Bacillus subtilis*, and preliminary and incomplete studies have been made on *Bacillus thermoacidurans*. In comparison with other aerobic spore formers, this latter organism is very reluctant to form spores and consequently almost any degree of sporulation has significance. Data are presented in general terms.

Effect of Carbohydrates on Sporulation

With the so-called Kahn strain of *subtilis* Perdue (1933) studied the effect on sporulation of several concentrations of glucose and lactose in peptone water. This organism did not metabolize lactose and as might be predicted lactose had no significant effect on sporulation. With glucose the results were quite different. A low (0.01 molal) concentration shortened the time of attaining maximum growth and sporulation, while a high (0.5 molal) concentration reduced both percentage and total spore yield. Roberts (1934) with the same organism, but with a synthetic base as a medium, found

that growth alone was favored by maltose and galactose, both growth and sporulation by levulose and to a lesser degree by sucrose and glucose, while trehalose, rhamnose, lactose, arabinose and cellobiose showed little or varied and less striking effects.

Hayward (1942) devoted some attention to the effect of carbohydrates on growth and sporulation of an organism designated as *B. subtilis* but not identical with that studied by Perdue and Roberts. He used acid hydrolyzed casein as a nutrient base, and compared xylose, arabinose, mannose, levulose, glucose, and the ketopentoses corresponding to xylose and arabinose. The ketopentoses were the most productive so far as growth was concerned. Fewer total spores and a lower percentage of spores occurred with xylose and xyloketose than with the other carbohydrates, which were essentially equal. From this fact Hayward suggested the possibility of a relationship between configuration and sporulation since the carbohydrates other than xylose and xyloketose have certain points of similarity in structure, and differ from xylose and its ketose derivative. This point needs much additional investigation.

Ward (1947) found glucose in a solid medium under aerobic conditions, and at 37° C. to be the most favorable of a number of carbohydrates for sporulation of *B. thermoacidurans*.

Studies on Synthetic Media

At the start of our work on spore resistance we expected to test the influence of various nutritive materials on the heat resistance of spores formed in the presence of the material. Toward this end we undertook to produce spore crops in simple synthetic media, but with complete failure. Good vegetative growth could be obtained, but microscopic preparations were negative for spores even after prolonged incubation. If the scarcity in the literature of references to sporulation in synthetic media is a criterion, others have had much the same experience.

Perdue undertook a study of the effect on growth and sporulation of different concentrations of several mineral salts as a preliminary to a reinvestigation of spore formation in synthetic media. She included both the chloride and the sulfate

of sodium, potassium, magnesium and ammonium, calcium chloride and ammonium phosphate. It would appear from her results that the sulfate ion, even in concentrations which restrain growth to some extent, has a decided stimulatory effect on sporulation. Only as potassium sulfate was there any delay in percentage sporulation, and here it was slight, and very probably due to a spore cycle in the medium. Phosphate appeared to stimulate both growth and sporulation at the levels tested, but in high concentration (2.5%) in synthetic media it was found by Roberts to be inhibitory to sporulation. The chloride ion seemed to have a decided inhibitory effect on spore formation, although in low concentrations it was possibly slightly stimulatory to growth. Among the cations ammonium and magnesium appeared to be definitely stimulatory to sporulation, as was calcium in a suitably low concentration. Sodium showed some evidence of a slight inhibitory effect. There was a definite indication that the early attainment of maximum growth, independent of the amount of growth, was accompanied by hastened sporulation.

Ward, also using a non-synthetic medium, has recently found that lithium, magnesium, calcium, iron, zinc, and manganese appeared to favor sporulation by *B. thermoacidurans* in media approximately neutral and incubated at 37° C. under aerobic conditions.

Roberts in a study of growth and spore formation in synthetic media (with asparagine as the source of both carbon and nitrogen) compared a considerable number of variations both as to mineral composition and concentration, all with microscopically negative results. A number of amino acids, both singly and in combination in the mineral base likewise were inadequate to induce more than a trace of sporulation and even this only in a few instances. Growth limitation without accompanying sporulation resulted if the amino acid concentrations exceeded certain critical levels—the level in the instance of cystine being as low as 0.1%. Likewise the incorporation in the medium of salts of a number of organic acids failed to afford an adequate nutritive environment for spore formation. Only with certain carbohydrates, especially fructose, as a supplementary carbon source could appreciable

sporulation be obtained, and sporulation was independent of the amount of growth. Modifications of a synthetic medium which allowed spore formation established that good sporulation would occur no matter what the source of nitrogen if fructose was supplied as a carbon source. Asparagine and several amino acids were available sources of both carbon and nitrogen for growth, and of nitrogen for spore formation if fructose were present in the medium. Asparagine and asparagine with added ammonium phosphate seemed to be superior to the amino acids alone as a nitrogen source for sporulation. As might be predicted optimum concentrations of the constituents of the synthetic medium could be established.

Effects of Growth Factors

The medium developed by Roberts was not satisfactory for spore formation by the organism studied by Hayward. Hayward attempted to remedy the deficiency by the addition of growth factors but with negative results. In a medium prepared from acid hydrolyzed casein he was likewise unable to improve sporulation by the incorporation of the growth factors studied, namely thiamin, nicotinic acid, pantothenic acid, biotin, pyridoxine, and riboflavin, although growth was stimulated to some extent. Inositol did increase slightly both the rate and the percentage of sporulation as well as the total growth. Ward found essentially no effect on sporulation by *B. thermoacidurans* under a variety of cultural conditions from any of eight water soluble growth factors. He also got no stimulation from a mixture of uracil, adenine, and guanine.

Miscellaneous Observations

Wynne (1948) has recently investigated the effects of several factors on sporulation by *Clostridium parabotulinum*, types A and B. In multiple strength brain heart infusion medium both growth and sporulation were depressed. In fractional strengths growth was depressed but percentage sporulation was increased. Enriching the basic medium by the addition of peptones did not appreciably alter the percentage of spores but did stimulate growth. Sodium chloride was found to have a depressing effect on sporulation much beyond its effect on

growth, while sodium phosphate depressed growth without affecting the percentage of spores. A comparison of the effects of differing concentrations of sodium chloride, potassium chloride and sodium sulfate showed essentially a straight line relationship between log of molar concentration of salt and log of number of total spores. His observations on the effect of sulfate on sporulation do not agree with those of Perdue or of Leifson.

Studies on Spore Resistance

Recently we have redirected our attention to heat resistance, with primary emphasis on fundamental factors which may be concerned. It has been known from the infancy of bacteriology as a science that spores of different species, and of different strains of the same species, might exhibit marked differences in heat resistance, but little or nothing is known as to why. We have considered first the possibility that there might be a difference in heat resistance among the vegetative cells which was transmitted to the spore. Zimmerman compared the resistance of vegetative cells and of spores of a number of aerobic spore formers. By repeated and frequent transfers it is possible to get a culture practically spore free. The number of spores, if any, can be easily determined by heating and plating an aliquot of a suspension, and future observations can take these data into consideration. Considerable differences in spore resistance were found among the organisms studied, and relatively great differences in vegetative cell resistance also. There was, however, no correlation. Low vegetative cell resistance might be associated with high spore resistance, and cultures showing high resistance for the vegetative cells might have spores of low resistance.

Davis (1948) has been able to increase by a considerable degree the resistance to heat of spores of *Bacillus globigii* by a process of repeated selection of survivors of exposure to boiling temperature. Thus we now have for comparative study two cultures which are descended from the same parent culture, and which regularly produce spores of appreciably different resistance to heat.

At the present time only a limited amount of work beyond the development of the two strains has been accomplished. It has been established that spore resistances to iodine and to phenol parallel heat resistance. Spore germination of each strain is similarly affected by a number of bacteriostatic agents, including among others crystal violet, penicillin, streptomycin, and mercuric chloride.

We think that these two strains in time will prove to be fruitful sources of information on the nature and mechanism of resistance not only to heat but to a number of other agents as well.

Summary

The results of studies on sporulation suggest that not all organisms are similarly affected by variations in the nutritive environment. Certain ions, and certain carbohydrates appear to be of more physiological significance than do others. Such growth factors as have been tested have not been observed to be significant.

It has been possible to develop by selection a strain of an aerobic spore former which gives spores having an appreciably enhanced resistance to heat. Further studies are in progress with this and the parent strain from which it was derived.

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MULTIPLE LYMPHOSARCOMATOUS ULCERS IN THE JEJUNUM: REPORT OF A CASE

R. H. Rigdon, F. F. Rogers, and O. T. Kirksey, Jr.*

Lymphosarcoma of the small intestines is a relatively rare lesion. However, according to Lieu (1925) it is the most frequent tumor in the small intestines. It occurs most often in the ileum and less frequently in the duodenum (Wallace, Miller, and Bell, 1942). The reticulum cell type is very rare; it occurred only three times in a group of sixty-nine cases reported by Frank and associates (Wallace, Miller, and Bell, 1942). Lymphosarcoma has been described both clinically and pathologically in the papers by Crowther (1913), Graves (1919), Ullman and Abehouse (1932), and Frank, Miller, and Bell (1942). This tumor may be either single or multiple (Weedan, 1929). Ewing (1928) states that "secondary tumors occurred almost exclusively in the intestinal mucosa, serous membranes and lymph nodes and were joined by continuous growths through lymph channels; two features distinguishing this process from true metastatic cancer." Kundrat (1893) considered the process not a spontaneous growth of multiple origin and with extension by metastasis but a regional disease of lymphoid tissue which propagated itself through the lymph path. Bell (1947) states that lymphosarcoma "develops as a localized growth in a lymph node or a mucous membrane and spreads by metastasis to the regional nodes."

The tumor mass in the intestines is usually single. The overlying mucosa may or may not be ulcerated. Frank and associates (1942) reported a case in which there were many small nodules in the submucosa. Ullman and Abehouse (1932) found fifteen cases of the annular or diffuse type of lymphosarcoma in a group of one hundred and twenty-five. This type involves more than one loop of the intestines. Graves (1919) reported a case of lymphosarcoma characterized by numerous ulcers in the jejunum, some of which extended down to the

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serosa. These ulcers frequently extended from the mesenteric attachment around the intestinal wall. Whitaker and Fisher (1934) described a case of lymphosarcomatous ulcer in the ileum that perforated.

The presence of either multiple nodules or ulcers in the mucosa of the intestines with involvement of the mesenteric and retroperitoneal lymph nodes presents a problem as to the site of origin of the tumor. The following case illustrates this relationship.

Report of a Case

Clinical Examination:

A sixty-five year old Mexican man entered the hospital because of abdominal pain that developed three months previously in the left upper quadrant. It was dull, aching in character, and was present constantly. The pain was aggravated both by food and lying flat in bed; it radiated through to the back. Since the onset of this illness the appetite was poor and there was a loss of twenty pounds in weight. There was neither nausea, vomiting, hematemesis, melena, nor any change in bowel habits. The past and family history were non-contributory.

The patient at the time of examination appeared chronically ill. The temperature was 98 degrees F., pulse 68, and respiration 20. The blood pressure was 140 mm. Hg. systolic and 82 mm. Hg. diastolic. The abdomen was scaphoid and approximately 4 cm. above the umbilicus and slightly to the left there was a firm, fixed, tender mass approximately 10 cm. in diameter. It did not move with respiration. A transmitted pulsation was very definite; however, no bruit was noted. The prostate was slightly enlarged.

The hemogram disclosed a red blood cell count of 4.44 M., a hemoglobin of 86 per cent and a white blood cell count of 10,100 with 80 per cent neutrophils. The total serum protein was 5.45 gms. per 100 cc. with 3.40 gms. of albumin and 2.05 gms. of globulin. A blood Kahn test was positive, Kolmer 2 minus, and a quantitative Kahn showed four units. The cerebrospinal fluid Wasserman reaction was negative. The sedimentation rate was 6 mm. in 30 minutes and 23 mm. per hour. A blood sugar was 97 mg. per 100 cc. Serum amylase

was 38 units per 100 cc. The prothrombin time was 77.7 per cent of normal. Cephalin flocculation was negative in twenty-four hours and plus-minus, forty-eight hours later. Occult blood was present in the stool. The urine showed a one plus albumin and 10–20 white blood cells per high power field.

A roentgenogram of the thorax revealed only a small nodule in the first interspace on the right. The heart was normal both in size and shape. Roentgenologic studies of the gastro-intestinal tract showed a normal filling, contour and pattern of the esophagus, stomach, and duodenum. The drainage was normal. An intravenous urogram revealed the calyxes, pelves, and ureters normal except for a slight displacement laterally of the proximal portion of the left ureter. A barium enema did not show any abnormalities in the colon. An oral cholecystogram was negative. A mild degenerative change was present in the lumbar vertebrae.

Five weeks following admission to the hospital the abdomen was explored under a general anaesthetic. A firm mass was found involving the mesentery of the small intestines and the retroperitoneal lymph nodes. The first portion of the jejunum showed several areas of transverse constricting lesions, "girdle ulcers," that completely encircled the lumen of the intestines. They resembled healed tuberculous lesions. The mucosa throughout the jejunum was hypertrophic. A large necrotic mass located at the duodeno-jejunal junction, continuous with the wall of the jejunum, was exposed for a biopsy. Due to the extreme friability of the intestines, the lumen was inadvertently entered. Because of this, it was necessary to resect this portion of the intestine with approximately 6 inches of the proximal portion of the jejunum.

The patient did poorly during the time of the operation and in spite of a blood transfusion expired shortly thereafter. The disease was considered to be either tuberculosis or lymphosarcoma while death apparently resulted from massive pulmonary atelectasis.

Pathological Examination:

The autopsy was performed three hours following death. Only the pertinent findings are included. The serosa of the small intestines was hyperemic and covered with a fibrinous

exudate. Approximately 100 cc. of a blood tinged fluid was present in the peritoneal cavity. A cavity-like area was present at the root of the mesentery adjacent to the ligament of Treitz. This was the site of the recent resection of a small portion of the jejunum. Many nodular masses of tumor cells were present in the mesentery. Some of the nodes measured 2 to 3 cm. in diameter and were hemorrhagic and soft, while others were grayish-white and moderately firm. Many of the blood vessels and lymphatics were surrounded by tumor cells and frequently their walls were infiltrated by similar cells. It was not uncommon to find the intima replaced by neoplastic cells. The lymph nodes around the head of the pancreas were infiltrated by the tumor. The periaortic lymph nodes from the level of the celiac axis to a point inferior to the bifurcation of the aorta also were markedly enlarged. Tumor cells infiltrated the adventitia of the aorta and the inferor vena cava. The ureters were pushed laterally by the tumor mass.

The most conspicuous lesion in this case was present in the jejunum. Firm, scarred masses of tissue were sometimes present in the mesentery at the attachment with the jejunum. The intestines in such areas showed the serosa to be hemorrhagic and scarred as though a narrow band had encircled the entire circumference. The width of such lesions varied from 1 to 2 cms. (Fig. C). Irregular ulcers were present in the mucosa of the jejunum corresponding to the areas of scarring on the serosa. Small masses of tumor cells protruded from the base of these ulcers. Sometimes the neoplastic cells extended through the muscularis to the serosa. The larger ulcers completely encircled the lumen of the jejunum. There were many smaller ulcers in the mucosa that were located away from the mesenteric attachment and were separated from each other by normal mucosa.

There were no lesions in any portion of the gastro-intestinal tract other than in the jejunum. One metastatic lesion was present in the lower lobe of the left lung. It was about 1 cm. in diameter, grayish-white in color and relatively firm. There was some scarring in the surrounding lung parenchyma. The tumor cells here varied widely in size and shape. Many were multinucleated. A portion of the wall of a small blood vessel

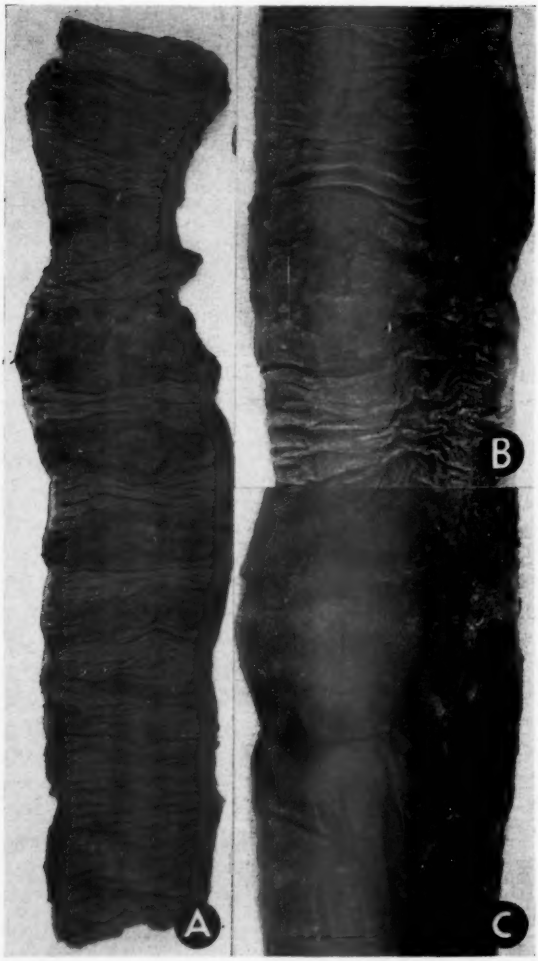

FIGURE A. The ulcers are numerous in the jejunum. The large ones completely encircle the lumen while others appear as small focal areas of necrosis in the mucosa. The base and walls of these lesions are infiltrated with lymphosarcomatous cells.

FIGURE B. The base of these large ulcers is irregular and relatively firm as the result of the presence of tumor tissue. The mucosa between the ulcers is normal.

FIGURE C. Tumor cells infiltrate the serosa over the large ulcers. The process is diffuse but should not be confused with a tubercular reaction.

was replaced by neoplastic cells. There was some hyperplasia of the vertebral bone marrow. In some areas we found oval and slightly spindle-shaped cells that resembled some of the cells found in the retroperitoneal lymph nodes.

Histologically the neoplastic ulcers in the jejunum were formed by reticulo-endothelial-like cells. These cells frequently extended through the wall and infiltrated the serosa. The cells which composed this neoplasm varied slightly in size in the ulcers and very greatly in size in the lymph nodes. In the latter there were numerous hemorrhagic and necrotic foci. A small amount of fibrosis was present in one of the lymph nodes. Sometimes the tumor cells were round with large nuclei and eosinophilic staining cytoplasm, while in other areas the cells were spindle in shape. Multinucleated cells could be found. The reticulum was most conspicuous in the lymph nodes and in the tumor tissue in the wall of the jejunum. The walls of some of the blood vessels located in these mesenteric lymph nodes were infiltrated by tumor cells. After studying all the lesions in this case it seemed to us wise to consider it as one of lymphosarcoma of the retroperitoneal lymph nodes with metastasis to the lungs and extension and multiple metastasis to the jejunum. Others may prefer to consider it either as Hodgkin's disease, since there was hyaline fibrosis and multinucleated cells, or as a reticulum cell sarcoma.

Anatomical Diagnosis:

(History of resection of portion of the jejunum and death on operating table.) Lymphosarcoma of retroperitoneal, mesenteric, pancreatic and periaortic lymph nodes; multiple ulcers in jejunum secondary to direct extension and metastasis from mesenteric and retroperitoneal lymph nodes; metastasis to lower lobe of left lung; questionable metastasis in vertebral bone marrow; mucous in bronchi; atelectasis of posterior portion of both lungs; old fibrous pleural adhesions, left upper lobe.

Discussion

The presence of multiple ulcers in the mucosa of the jejunum and marked lymphadenopathy of the adjacent lymph nodes first suggested an infectious process; however, on cut section

the nodes appeared to be grossly those of a lymphosarcoma rather than those of tuberculosis. Furthermore, no tubercles were present on the serosal surface of the jejunum. Hayden and Apfelback (1927) reviewed the problem of lymphogranulomatosis of the gastrointestinal variety and stated that "the difference of opinion among pathologic anatomists in regard to the anatomic classification of lymphogranulomatosis proves that the histologic interpretation of this disease is also deceptive. There are instances in which there is a single tumor of the bowel, and others with extensive and multiple ulcers of the gastro-intestinal tract alone, or associated with enlargement of several groups of lymph glands." Our case easily could be put into this group. However, the absence of eosinophilic leukocytes, plasma cells and the infrequency of multinucleated cells within the gastro-intestinal tract does not suggest to us a Hodgkin process. Furthermore, the lesions in the mesenteric nodes and the lung resembled the so-called Hodgkin's sarcoma more than the granulomatous form of the disease. The diagnosis of lymphosarcoma in our case has been made reluctantly. However, it seems to be consistent with the discussion of lymphosarcoma by Sugarbaker and Craver (1940).

The clinical findings in this case of lymphosarcoma of the small intestines are similar to those described by others (Wallace, Miller, and Bell, 1942, and Weedan, 1929). A majority of the cases occur, however, at an earlier age than did this one. The type of ulcers and their distribution are similar to a case reported by Graves (1919) who considered the lesions to be primary in the intestine. He did not discuss, however, the problem whether the lesions were multiple in origin or whether they represented metastatic growths. Weedan (1929) considered his case (No. 4) to be one in which the lymphosarcoma was primary in the lymph nodes with metastatic nodules in the mesentery of the small bowel and one large tumor in the ileum which probably was metastatic. Ewing (1928) has classified one type of lymphosarcoma as retroperitoneal. "It produces a large abdominal tumor composed of fused lymph nodes, while the extensions may be either very wide or rather limited. Such tumors may be associated with

intestinal lesions so that it is difficult to determine the primary seat."

The presence of many excessively large nodes in the area of the head of the pancreas, retroperitoneally and along the aorta in our case, would suggest that probably the tumor had been present for a longer time in these nodes than in the mucosa of the jejunum. Furthermore, in support of this opinion it may be stated that the lymph nodes in the periphery of the mesentery were enlarged as the result of the presence of tumor tissue but they were not as large as those farther up in the group. If the lesion was primary in the jejunum we would expect the nodes closest to the primary growth more likely to be larger than those more distal. Likewise, the multiplicity of lesions in the intestine suggested to us that they more likely developed as a result of an extension of tumor cells either down the mesenteric lymphatics or as emboli through the branches of the mesenteric arteries than that they originated from multiple primary foci within the mucosa or submucosa. The wall of some of the blood vessels in the tumor mass was infiltrated with neoplastic cells—from such areas tumor emboli could have originated.

It is difficult in our case to explain the mechanism of restriction of the ulcers to the jejunum. Lymphosarcoma originates in lymphoid tissue and it is thought to arise more frequently in the ileum than in the jejunum because the quantity of lymphoid tissue is greater in the former. It is suggested that the localization of the ulcers to the jejunum in this case is merely a coincidental finding. The lymphatics in this portion of the mesentery apparently were involved earlier than those draining the ileum. No doubt similar ulcers would have developed in the mucosa of the ileum had the patient survived for a longer time.

Summary

A case is reported of lymphosarcoma involving the mesenteric, retroperitoneal and periaortic lymph nodes with multiple lymphosarcomatous ulcers in the jejunum. It is suggested that the ulcers are secondary to the tumors in the lymph nodes. The variations in the histological structure of the tumor in different areas makes a diagnosis difficult.

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A TRANSPLANTABLE FIBROSARCOMA IN RATS

C. P. Coogle and R. Lee Clark, Jr.*

During a period of approximately three years we have observed a transplantable fibrosarcoma in rats of the Wistar strain. This tumor, which originally occurred spontaneously in the subcutaneous tissue of an albino rat of uncertain stock, has now been carried through twenty-seven transplantation generations. It grows regularly and successfully in animals of the Wistar strain and constitutes in our laboratory a readily available reserve of test material. Some use of this material has already been made, and we now propose to set up a series of experimental conditions designed to demonstrate fully its biological properties. The present report is intended only to outline the history of the tumor and to describe its gross and microscopic pathology.

The tumor is referred to in this laboratory as the A H tumor in deference to the M. D. Anderson Hospital for Cancer Research where these observations have been made.

History of the Tumor

The A H tumor was found on February 8, 1945, as an apparently spontaneous growth in the subcutaneous tissue of a male albino rat. This animal was approximately three months old at the time the tumor was noted. The first seven transplantation generations were made into animals of the same albino stock, after which the tumor has been carried in rats of the Wistar strain.

Methods of Transplanting

Although the tumor grows readily in any subcutaneous area, the back of the rat is chosen as most suitable for the planting. A small spot on the midback region is shaved and cleansed with acetone. After anesthetizing the animal, an incision about one-quarter of an inch in length is made through the skin and a sterile glass rod is forced beneath

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the skin up to the interscapular region. This channel quickly fills with blood and tissue fluid. A small piece of freshly cut tumor tissue is forced well up into the channel. No suture is used on the incision, but it is allowed to close itself or form a drain in event of infection.

At least ninety per cent of transplants are successful. The age or sex of the rats appears to bear no relation to the success of transplantation. Failures are usually due to infection in the transplantation site.

Gross Pathology

Almost immediately after transplantation the tumor begins to grow, and nodules three to five millimeters in diameter are developed within the first week. Maximum growth is attained in about sixty days at which time the tumors may weigh as much as 125 or 140 grams. Many of the tumors weigh more than the animal host. The growth of the neoplasm tends to extend symmetrically with the axis of the animal body, confined at all times in a firm fibrous capsule. The tumors are not attached to the deeper structures and do not penetrate the body cavity directly. The smaller tumors are soft or rubbery in consistency, but become quite firm as maximum size is approached. Occasionally the tumors are finely nodular, and at other times a single mass is formed. The sectioned surface demonstrates a dense fibrous capsule and trabeculae separating the various nodules. Central areas of softening, hemorrhage, and necrosis are common. The surface of the tumor itself is rather dense and pearly white or slightly pink.

Microscopic Pathology

In microscopic section the predominant cell of the tumor is a large fibroblast. These vary somewhat in size, but as a rule are rather uniform. There is some irregularity in nuclear form and staining density, and a few mitotic figures are seen. For the most part the cells are arranged parallel to one another in interlacing bundles. The amount and density of intercellular material varies rather widely in sections taken from different tumors. The capsule is always intact, and local

invasion or metastatic growths have not been demonstrated. A typical microscopic section is shown in the accompanying photograph.

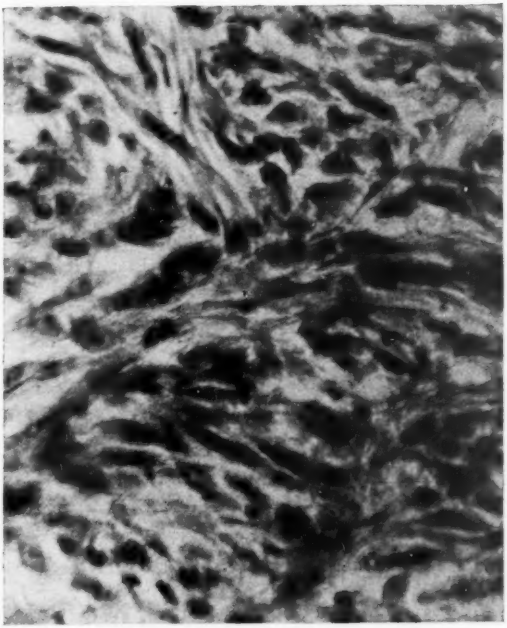

FIG. 1. Microscopic section of the transplantable fibrosarcoma.

Comment

The rapid growth and ease of transplantation suggest that this tumor be classed as a malignancy. Most of the microscopic sections show more cytologic pleomorphism than would be expected in a benign fibrous neoplasm. A constantly intact capsule, lack of invasion or metastasis, failure to recur after complete removal, and absence of severe generalized toxic reaction in the animal host are features which usually characterize a benign growth.

Our interest in this tumor is held by the fact that it is a readily available and easily controlled source of neoplastic tissue for experimental observation.

Summary

We have noted the spontaneous occurrence and twenty-seven transplantation generations of a fibrosarcoma in rats. This neoplastic tissue is being subjected to observations, the results of which will be reported later.

PENICILLIN IN PRENATAL SYPHILIS

H. F. JOHNSON AND C. N. FRAZIER*

It has been estimated that each year in this country 85,000 pregnancies result in infection of the fetus with syphilis (Cole, 1940). In recent years there has been a general reduction in the incidence of syphilis on most obstetrical services, indicating that the above figure was probably even higher only a few years ago (Stokes, 1944). This gradual reduction may be attributed to the added emphasis on the control and prevention of syphilis in the general population through routine serologic examinations of larger groups of people (food handlers, applicants for employment or marriage, etc.), better knowledge of the disease, and improvements in therapy.

Among these advances, the introduction of penicillin stands foremost in recent years. By offering a form of treatment relatively simple in administration, almost free of severe side reactions, and achieving maximum protection with courses of treatment of short duration, this drug has overcome most of the objectionable features which previously plagued both physician and patient.

The prevention of prenatal syphilis still rests primarily on careful premarital examinations and on constant vigilance toward the early detection and treatment of the disease in pregnant women. The term "constant vigilance" should be emphasized strongly, since syphilis may be contracted at any period during the pregnancy. It has been shown that the bloodstream may be infectious during the seronegative phase of syphilis (Frazier), thus demonstrating the fallacy of depending upon a solitary examination, especially early in pregnancy, to rule out this disease.

Goodwin and Moore (1946) estimate that, with the best metal chemotherapy, approximately 5 per cent of infants born of mothers with primary or secondary syphilis are infected, and that, with less adequate amounts of treatment, 30

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to 50 per cent of the infants may be infected. The unpleasantness of this type treatment and the prolonged periods required for its administration, often lead to disruption of the therapy before sufficient amounts can be given. For this reason it is apparent that a large percentage of the mothers fail to receive "adequate treatment," and consequently fall into the higher bracket of failures of pregnancy due to syphilis.

Recent investigations appear to show that penicillin is especially effective in protection of the infant from infection in the presence of syphilis in the mother. A composite survey of five such reports covers 493 pregnancies (Ingraham, 1947), Goodwin and Moore (1946), Speiser (1947), Aron (1947), and Olansky (1947). Thirty-seven resulted in fetal deaths, four being labelled as possibly syphilitic in origin. This incidence is within the range anticipated in the general population. Only eight, or 1.8 per cent, resulted in disease in the infant. In four of these cases, the mothers subsequently were treated for infectious relapse. Contrary to experience with older conventional types of therapy, several mothers were effectively treated in the last weeks of pregnancy, suggesting that a portion of these failures might have been averted had it been possible to recognize and retreat the relapses prior to delivery.

It seems worthwhile to add to this number our experience with 60 pregnancies at John Sealy Hospital of The University of Texas. Twenty of these have been reported previously (Frazier, 1946). A total of 82 pregnancies were observed, but 17 had to be excluded because of lack of data on the infants. Five additional patients received no treatment prior to delivery.

Method of Study

Early infectious patients were used principally, though several latent cases were included. Some were patients previously treated with penicillin who subsequently became pregnant. These were not treated during pregnancy. The others were found to have syphilis and were treated in the course of their prenatal care.

Penicillin was administered in schedules varying from 0.6 million units to 4.8 million units of amorphous sodium or

crystalline "G" penicillin. An additional schedule consisting of 9.6 million units of penicillin in wax and oil, given in doses of 600,000 units twice weekly, was used in some cases.

Efforts were made to examine the mothers monthly during pregnancy. When possible, the infants were seen monthly for three months, and again at six, nine, and twelve months. Quantitative serologic tests by Kahn or Kolmer techniques were used. No additional treatment was given to the mothers after the original course, unless there was evidence of clinical or serologic relapse, or a serologic reaction of high titer was maintained for one year after treatment.

General Results

Of sixty pregnancies, six terminated disastrously. No examinations were made of the fetal remains, but three were felt to be almost certainly unrelated to syphilis. The remaining three were interpreted as possibly syphilitic in origin. In the latter group, one stillbirth occurred at seven months during treatment of the mother. In the other two cases, with fetal deaths occurring at six months and eight months in gestation, the mothers subsequently were retreated for serologic relapse.

One infant was born with syphilis. The mother had a serologic relapse at term, but because of a gap in observation, was not treated prior to delivery. The infant's serologic titer fell from 1:64 at birth to 1:16 (Kahn) by the 35th day, and there was no clinical evidence of infection. The child did not return until the 70th day, at which time the Kahn test was 1:512 and examination revealed snuffles, hepatitis, and splenomegaly. Treatment with penicillin has given an apparently satisfactory response.

The six fetal deaths represent a 10 per cent incidence of failure of pregnancy—in the range of anticipated failures among all pregnant patients (13.6 per cent at John Sealy Hospital in 1947). The one infected infant represents a failure rate among the living infants of 1.8 per cent. Moreover, it is felt that this failure could have been prevented if the stipulated monthly observations had been made.

Of particular interest are five normal infants born of mothers with early syphilis treated during the last month

of pregnancy. A total of fifteen women with early syphilis were treated during the last trimester of pregnancy, all giving birth to normal infants. From this, it would appear that penicillin exerts a curative effect on the infected fetus in utero, as well as on the disease in the mother. In fact, when the prescribed schedule of observations was followed by the prospective mother, only one fetal death occurred and that was of questionable syphilitic origin.

Ten multiple pregnancies were seen. Six mothers in this group received no further treatment after the initial course. The remaining four were retreated for serologic or clinical relapse—three during, and one prior to the second pregnancy. In every case the second pregnancy produced a normal infant. Three of the retreated mothers had a positive serology at time of delivery. In two of these cases this was seven and eight months after treatment. In the group not retreated, at the time of delivery, two had weakly positive serologic tests, fourteen and twenty-one months after treatment. This would tend to contradict the concept that all women with treated syphilis should be retreated during successive pregnancies. The birth of normal infants by twenty-five patients treated prior to pregnancy lends further support to this view. This prolonged protection seems to suggest a curative rather than a suppressive effect by penicillin on the disease in the mother.

Results by Amount of Penicillin

In the case of the one failure, the mother had received 4.8 million units of penicillin for secondary syphilis. The occurrence of a serologic relapse during the pregnancy indicates a failure of penicillin to exert a curative effect on this patient. The fetal deaths all occurred among patients treated for early syphilis, one with 1.2 million units, two with 2.4, and three with 9.6. Two occurred during treatment, one at three months in gestation, and one at seven months. Thirty-five mothers were treated during pregnancy, yielding a 5.7 per cent incidence of abortions questionably related to the penicillin therapy.

The individual groups by amount of penicillin received are too small for statistical analysis, but it appears that protec-

tion of the infant does not depend too much upon the amount of treatment. However, it is probable that 1.2 million units is the minimal satisfactory dose.

Five patients received no penicillin prior to delivery. Two resulted in syphilis in the infant. In these cases the mothers had received no previous treatment of any type and diagnosis was made during the obstetrical admission. Both infants had a negative serologic test at birth, but developed lesions of syphilis at 34 and 75 days. The remaining three patients had received metal therapy in the past, but still retained positive serologic tests. One five months abortion of unknown cause and two normal infants resulted.

Results by Stage of the Disease

Patients with primary or secondary syphilis, and three patients with latent syphilis in whom the disease had been present for two, six and eleven months at the time of treatment were considered to have "early" syphilis. Those with syphilis existing over one year, or of unknown duration, were classified as "late" syphilis. There was one failure in forty-eight among the early, and none among the late. Five of the six disastrous results were among the early cases. The mother of the infected infant and four of the patients having abortions were treated prior to pregnancy. Two of these fetal deaths were considered possibly syphilitic, as compared to one among the patients treated during pregnancy.

Cord Blood

Positive serologic tests on the cord blood was found in six cases. Twenty-three gave negative results. The six infants, with positive serologic tests varying in titer from 1:10 to 1:320 (Kolmer), all gave negative tests for syphilis after periods ranging from six to eighteen weeks. The average time required to attain negativity could not be estimated because of insufficient observations. The cord blood of the infected infant was negative at birth, and a positive reaction was not found until several weeks later, suggesting that serologic tests in the neonatal period may be misleading, and that serial testing should be done in all suspicious cases for a period of at least three months.

Follow-up Examinations of Infants

Among the infants classified as normal, thirty-three were followed six months or longer, eleven for twelve to twenty-six weeks and nine for less than twelve weeks at the time of this writing. Of the nine inadequately followed infants, three were serologically negative at four weeks, one at six weeks, two at eight weeks, and three at ten weeks. At delivery in every case the mother's blood was negative, or positive in less than three tubes. It is felt most unlikely that any of these might later develop syphilis.

Course of Patients Treated

Altogether, sixty women have been treated with penicillin. Twenty-five have reached and maintained a state of seronegativity, and twenty-four show a marked reduction in titer to borderline levels (1:40 Kolmer and 1:4 Kahn or less). Only three retained a moderately high titer, two of these being in the late latent group. Eight patients relapsed and were retreated. Two of these have become seronegative and five have attained a borderline titer. No observations are available on the remaining case.

Reactions

Except for an occasional Jarisch-Herxheimer response no reactions to the therapy were noted in this series. Local pain at the site of the injection was often present, but was tolerated well by alternating the site of the injection. In the past two years, among all classes of patients receiving penicillin at John Sealy Hospital, we have seen only one severe case of dermatitis attributed to penicillin. Urticarial reactions have been uncommon, and these have responded well to anti-histamine drugs. In several such cases, where it was deemed advisable to continue the penicillin, the urticaria subsided and no apparent ill-effects were encountered as long as the anti-histamine preparations were continued.

Summary

Of sixty syphilitic pregnant women treated with penicillin, one gave birth to a syphilitic infant. Fetal deaths were within the range of anticipated failures of pregnancy.

Following one course of the treatment, protection appeared to extend to subsequent pregnancies, obviating the need for additional treatment.

These findings are in agreement with the published reports of studies along similar lines made at other institutions.

Conclusion

Penicillin appears to be the best available form of therapy for syphilis in pregnancy from the standpoint of protection of the infant, even in the last stages of pregnancy, ease of administration, shortness of the course for effective results, relative absence of reactions to the drug, and permanent cure for the mother.

Table I

Results of Treatment
Comparative Survey of Various Groups

Groups	Number of Pregnancies	Fetal Deaths		Number of living Infants	Infants with Syphilis	
		No.	%		No.	%
Philadelphia (Ingraham 1947)	142	12	8.4	130	4	3.1
New York (Speiser 1947)	261	23	8.8	237	4	1.7
Chicago (Aron 1947)	36	2	5.5	34	0	0
Baltimore (Goodwin 1946)	33	0	0	33	0	0
Washington (Olansky 1947)	21	2	9.5	19	0	0
Galveston	60	6	10.0	54	1	1.8
TOTAL	553	45	8.1	508	9	1.8

TABLE II

Results of Treatment According to Penicillin Dosage

Amount of Penicillin	Early Syphilis in Mother				Late Syphilis in Mother			
		Results of Pregnancies				Results of Pregnancies		
	No. of Pregnancies	Normal Infants	Fetal Deaths	Infants with Syphilis	No. of Pregnancies	Normal Infants	Fetal Deaths	Infants with Syphilis
0.6 Million Units	6	6	....	....	....	....	....	—
1.2 Million Units	16	15	1	....	3	3	....	—
2.4 Million Units	9	7	2	....	6	6	....	—
4.8 Million Units	10	9	....	1	1	1	....	—
9.6 Million Units	8	5	3	....	1	1	....	—
TOTAL	49	42	6	1	11	11	0	0

TABLE III

Results According to Time of Treatment in Relation to Pregnancy

Stage of Disease in Mother	Time of Treatment	No. of Pregnancies Observed	Cord Blood			Results in Infants		
			STS +	STS —	STS No Data	STS —	Fetal Deaths	Infants Infected with Syphilis
Early	Treated during Pregnancy	23	3	10	9	22	1	0
	Treated prior to Pregnancy	25	0	11	10	20	4	1
Late	Treated during Pregnancy	12	3	1	7	11	1	0
	Treated prior to Pregnancy	0	0	0	0	0	0	0
TOTAL		60	6	22	26	53	6	1

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A MODIFICATION OF THE ILIOFEMORAL EXPOSURE OF THE HIP JOINT

G. W. N. EGGERS*

The iliofemoral exposure of the hip joint (Bardenheuer, Sprengel, Larghi, Smith-Petersen) has been altered in various ways to conform to the surgeon's specific purposes. The length of the incision, degree of exposure, division of the straight head of origin of the rectus femoris, and other modifications are accepted technical procedures. The modification presented has been useful within the limitations and the purpose for which it is intended.

A problem which presented itself in our work on the hip joint was the pain and persistent flexion following vitallium cup arthroplasties of the hip in the cases which we considered unsatisfactory. After proper physiotherapy and interval of time with no improvement, we were permitted by some of the patients to investigate these hips surgically. In all of them we found dense cicatricial tissue which formed anterior to the neck of the femur and which was of definite hindrance in extending and laterally rotating the hip joint. Removal of the scar mass was comparatively easy, but when completed, we had no assurance that it would not recur; in fact, felt certain it would. To eliminate this so-called "dead space" was decided as the best solution to the problem. To accomplish this, the straight head of origin of the rectus femoris was detached from the acetabulum, close to the bone to secure the strong tendinous origin. The straight head was then displaced laterally and sutured to the superior posterior margin of the iliofemoral ligament of the hip joint. Thus the neck of the femur was covered by the muscle and the "dead space" in proximity to the hip joint eliminated, preventing future anterior excessive cicatricial tissue formation, which would re-

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strict hip joint extension and lateral rotation. The elasticity of the muscle fibers longitudinally and transversely permit freedom of motion.

The modification described has also been useful in removal of scar tissue in old infected hip joints where the mass was anterior and adjacent to the hip joint.

The transplantation of the straight head of the rectus femoris origin to the upper margin of the ligament we now use routinely in two types of hip joint conditions: 1. When in the judgment of the surgeon the iliofemoral ligament is removed for operative expediency. 2. After removal of excessive scar tissue anterior to the hip joint which usually involves the iliofemoral ligament.

The transference of the straight head does not affect the stability of the knee joint; hip joint flexion may be slightly weakened temporarily and perhaps is desirable in flexion cases.

Flexion of the knee must be prevented by Buck's traction or a light posterior knee splint for about two weeks after operation to assure firm union of the transplanted straight head of the rectus femoris.

The modification of the iliofemoral incision described is for the purpose of retaining or improving hip joint function by decreasing restricting cicatricial tissue formation adjacent and anterior to the hip joint.

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FIGURES I to IV. Dissection to show anatomy of incision.

FIGURES V–VI. Operative modification; iliofemoral ligament (6) removed.

1. Sartorius.
2. Rectus femoris, straight head, origin.
3. Tensor fascia femoris.
4. Rectus femoris, reflected head, origin.
5. Lateral circumflex artery and vein.
6. Iliofemoral ligament (fat removed).
7. Iliofemoral ligament, superior-posterior cut margin.

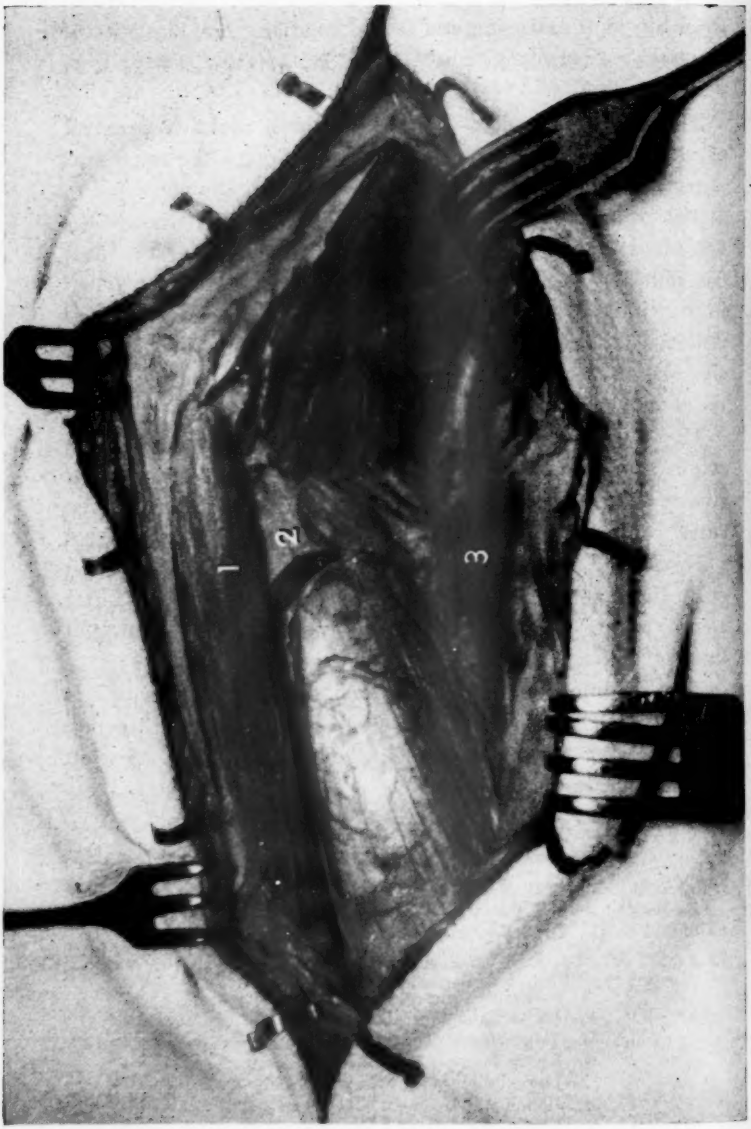
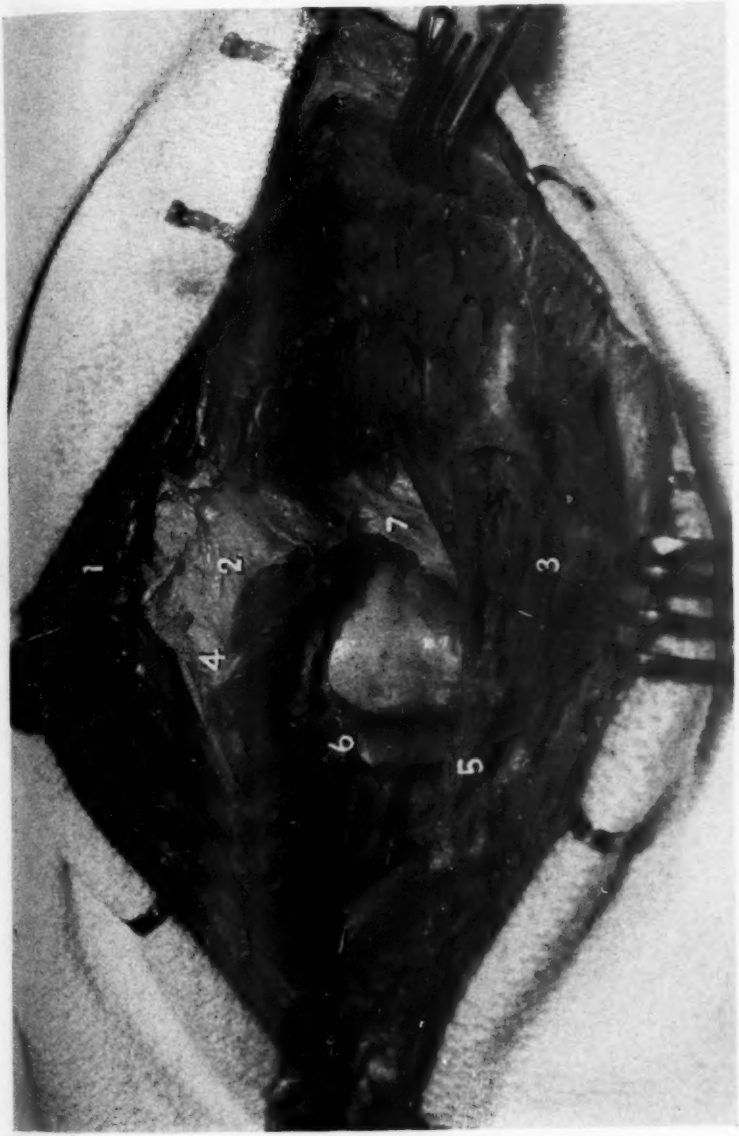


FIG. 1.

FIG. 2.

FIG. 1.

Fig. 3.



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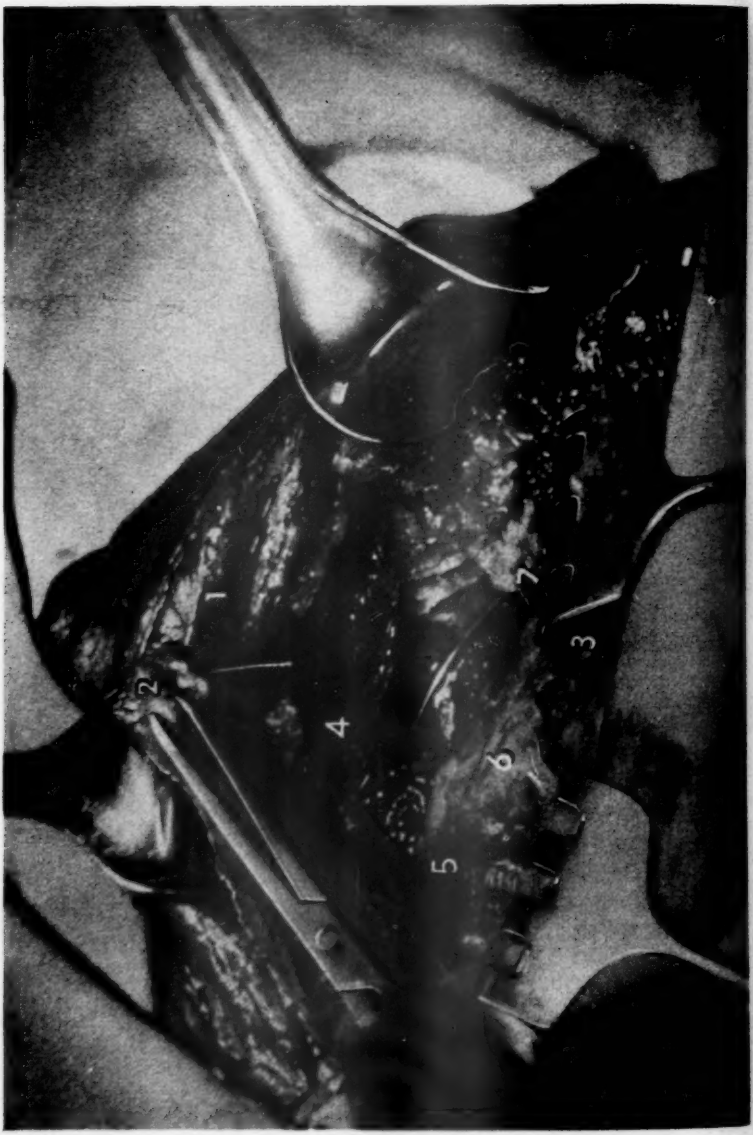

FIG. 5.

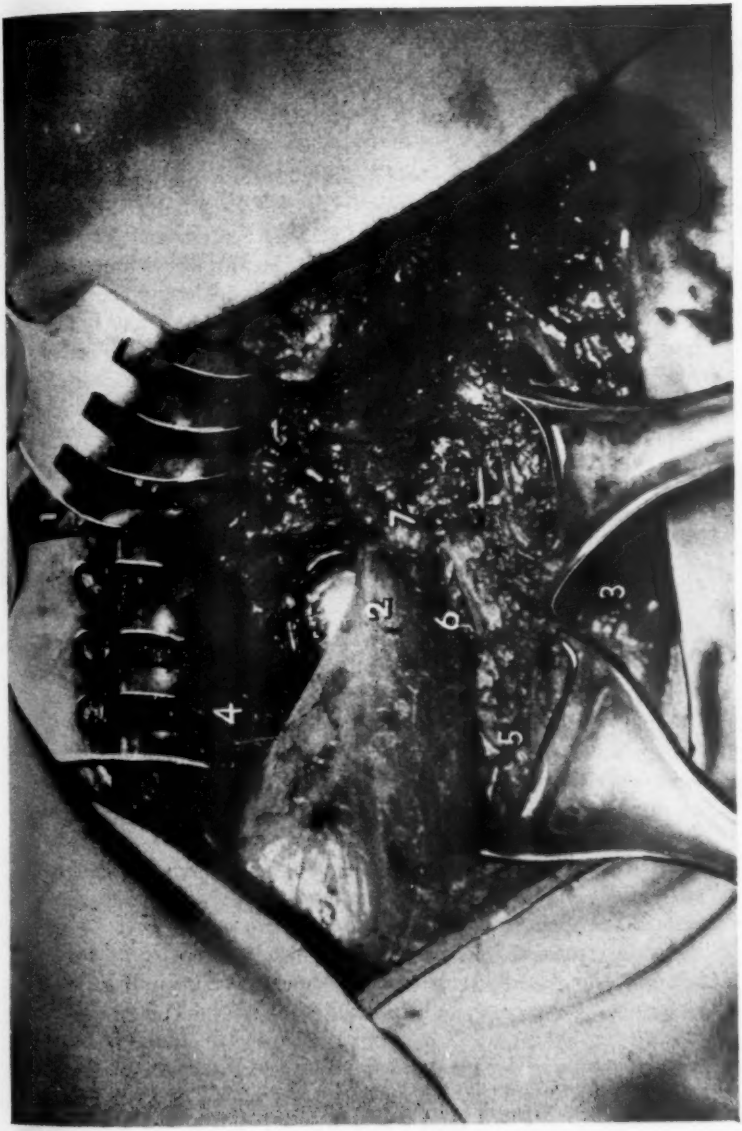

FIG. 6.

ENTOMOLOGICAL STUDIES ON TYPHUS IN LAVACA COUNTY, TEXAS

R. M. Randolph, L. J. Ogden, and R. B. Eads*

This paper presents a description and discussion of the entomological studies on endemic typhus conducted in Lavaca County, Texas, from October, 1945, to September, 1946, by personnel of the United States Public Health Service and the Texas State Department of Health. This work was a continuation of that begun by Rude and Davis (1945).† These studies had the following objectives: (1) determine the occurrence of the various species of ectoparasites on common domestic rats; (2) continue studies of the effectiveness of DDT in reducing populations of rat-ectoparasites in urban establishments; and (3) evaluate in rural areas the effect of DDT when dusted on rat runs on the rat-ectoparasite populations.

Part 1

The Occurrence and Abundance of Species of Ectoparasites on the Domestic Rat

Abundance records for rat ectoparasites were obtained from *Rattus rattus* trapped in the town of Hallettsville, Lavaca County, Texas, during the period from October 1, 1945, to September 1, 1946. No rat or ectoparasite control measures were carried out in order to prevent upsetting the normal occurrences of ectoparasites and rats in this town. Rats were trapped in all types of buildings in urban areas, including feed stores, warehouses, grocery stores, cafes, residences, and out-buildings. Number "0" steel traps were used and the tension of the springs was set so that the trap would hold the rat alive since most ectoparasites leave the rat soon after its death. Traps were placed in rat runs in the afternoon and the rats caught were removed early the following day. The rats were

*From the Bureau of Laboratories, Texas State Department of Health, Austin. Received for publication July 29, 1948.

†Rude, C. S., and D. E. Davis. The use of DDT for the control of ectoparasites on rats. Unpublished manuscript, 1945.

placed in flea-tight cloth bags and brought to the laboratory where they were bled for serological examination and combed for ectoparasites. In order to obtain live ectoparasites, a rat, if not killed during the bleeding, was stunned by a blow and placed in a deep white enamel pan for combing. Since combing frequently did not remove all the parasites it was usually necessary to hold the rat by the tail and give it a few sharp blows with a stick. Very good results also were obtained by examining the fur of the animal closely and removing ectoparasites with a pair of forceps. Live ectoparasites were taken from the rats, identified and sorted according to species. Specimens of each species were pooled and placed in a sterile test tube, sealed under glass and frozen for future epidemiological studies. After live ectoparasites were obtained for the epidemiological studies, the rats were chloroformed and reexamined for parasites that might have escaped notice previously. Approximately twenty live rats were trapped each month from establishments in Hallettsville.

In this study the following species of ectoparasites were found to occur on the common domestic rat, *Rattus rattus:*

SIPHONAPTERA, Fleas

Xenopsylla cheopis (Rothschild)
Leptopsylla segnis (Schonherr)
Echidnophaga gallinacea (Westwood)
Pulex irritans Linnaeus
Ctenocephalides felis (Bouche)
Nosopsyllus fasciatus (Bosc)

ACARINA, Mites

Liponyssus bacoti (Hirst)
Echinolaelaps echidninus (Berlese)
Laelaps nuttalli Hirst
Eulaelaps stabularis (Koch)
Dermanyssus gallinae Redi
Atricholaelaps megaventralis Strandtmann

ANOPLURA, Lice

Polyplax spinulosa (Burmeister)

The abundance of the more common species of ectoparasites is shown in Table I. It can be seen that the highest incidence of the tropical rat flea, *Xenopsylla cheopis*, occurred during June, July, and August. The mouse flea, *Leptopsylla segnis*, was most abundant during March and April. The table also shows that a high percentage of the rats was infested with fleas throughout the year. Mites and lice were also found on rats during almost every month of the year. However, the percentage of the rats infested with mites and lice was found to be lower than that of rats infested with fleas.

TABLE I

Seasonal Variation of the Most Common "Rattus rattus" Ectoparasites During the Period October 1, 1945 to September 1, 1946, Hallettsville, Texas

Month	No. of Rats examined	Average No. of *X. cheopis* per rat	Average No. of *L. segnis* per rat	Average No. of *E. gallinacea* per rat	Average No. of Fleas per rat	Percent Rats Infested with Fleas	Average No. of *L. bacoti* per rat	Average No. of *L. nutalli* per rat	Average No. of Mites per rat	Percent Rats Infested with Mites	Average No. of *P. spinulosa* per rat	Percent Rats Infested with Lice
Oct. 1945	24	2.3	0.5	0.3	3	66.7	5.0	1.3	6.3	54	2.0	29
Nov. 1945	32	4.8	5.3	0.5	10.6	90	10.0	0.3	10.3	63	2.8	63
Dec. 1945	14	2.9	1.2	0	4.1	86	,0.1	0	.7	7	1.8	64.3
Jan. 1946	3	1.3	3.0	0	4.3	100	0	0	0	0	6.3	33.3
Feb. 1946	8	2.3	1.1	6.1	15.6	100	1.6	0	1.6	50	7.1	50
Mar. 1946	18	1.4	19.0	0.8	21.2	100	0.2	0	.2	16.7	1.5	39
Apr. 1946	17	4.4	6.1	0.5	11	100	3.7	0	3.6	23.5	3.9	17.6
May 1946	19	6.1	1.9	0.7	8.7	90	4.7	0.1	4.8	26.3	6.9	52.6
June 1946	20	8.0	1.4	0.3	9.7	90	1.5	0	1.5	25	1.4	25
July 1946	19	13.0	0.1	0.6	13.7	94.7	0.3	0	.3	10.5	0.6	20.1
Aug. 1946	20	10.4	0	.3	10.6	90	.05	0.05	.1	10	2.1	35

Part 2

DDT Studies in Urban Areas

The original work on the effectiveness of DDT for the control of rat ectoparasites was done by Davis (1945) and by Rude and Davis (1945). The present study covers the period October, 1945, to September, 1946, inclusive.

In Lavaca County, Texas, the towns of Moulton and Shiner were treated, and Hallettsville was used as a control. All three towns are close together and comparable in many respects. The records of rat-ectoparasite populations obtained in Hallettsville as described in Table I are compared with records of rat-ectoparasite populations obtained from Shiner and Moulton by similar methods during the same period. As in the original work a monthly collection was made of ten rats each from Moulton and Shiner and twenty rats from Halletsville.

DDT was applied as a 10% dust in pyrophyllite. The amount of dust used for each premises varied according to the size and type of establishment, the number of actual and potential rat runs and the degree of rat infestation. Approximately one-half pound of dust was applied to small cafes and other small establishments, one-quarter pound or less for rat-infested areas in and around residences and two to eight pounds for large business establishments such as gins, mills, and large warehouses. The dust was applied by the use of Hudson Admiral hand dusters No. 775 and shaker-type hand dusters made by perforating the lids of pint jars. The dust was applied to potential as well as to actual rat runs. The monthly indices of *X. cheopis* in the check town of Hallettsville and the treated towns of Shiner and Moulton are compared in figures 1 and 2.

The application of 10% dust to rat runs in Shiner establishments resulted in much lower indices of *X. cheopis* and *L. segnis*. Results were less satisfactory in Moulton but indices of the above two species were significantly reduced by the application of the 10% DDT to rat runs. Low *Echidnophaga gallinacea* indices in both check and treated towns make it difficult to evaluate the effect on this flea of dusting rat runs with DDT. No significant differences between mite and lice populations on rats occurred in check and treated towns.

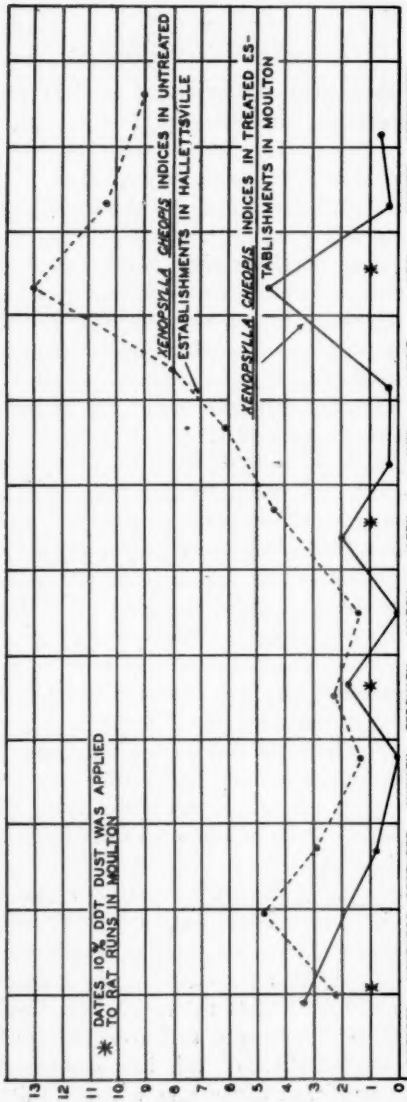

FIG. 1 COMPARISON OF *XENOPSYLLA CHEOPIS* INDICES IN UNTREATED ESTABLISHMENTS IN HALLETTSVILLE AND IN ESTABLISHMENTS TREATED WITH 10% DDT DUST IN MOULTON.

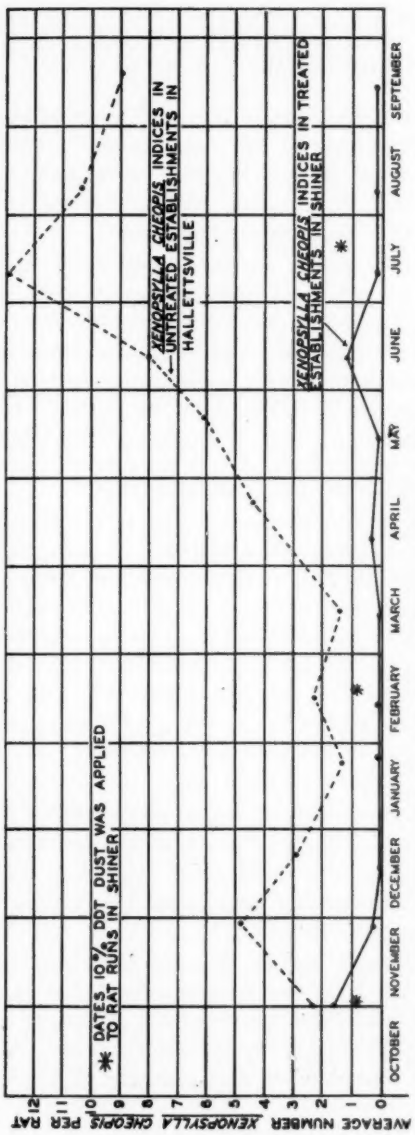

FIG. 2 COMPARISON OF *XENOPSYLLA CHEOPIS* INDICES IN UNTREATED ESTABLISHMENTS IN HALLETTSVILLE AND IN ESTABLISHMENTS TREATED WITH 10 % DDT IN SHINER

Part 3

DDT Studies in Rural Areas

In February, 1946, a study was instigated to evaluate the effect of DDT dusting of rat runs on the rat ectoparasite populations in rural areas. The rural investigation was motivated by the fact that at least half of the endemic typhus cases in the county occurred in rural areas.

This study was carried on simultaneously with the urban DDT dusting program. Thirty-two farms were tentatively selected for experimental purposes. Rats on these farms were trapped and examined for ectoparasites to determine if the ectoparasite populations were sufficient to warrant dusting. The original number of thirty-two was reduced to twenty-four because the amount of time consumed in trapping rendered the first figure impractical. Rats were trapped and ectoparasites were collected as previously described. A goal of three rats per farm per month was set. The rather low figure was chosen in order to prevent the depletion of the rat populations by trapping. Of the twenty-four farms, six were selected at random as checks and were not dusted. The other eighteen farms were dusted with 10% DDT in pyrophyllite after initial trapping whenever the rat-ectoparasite count on these farms increased sufficiently to warrant treatment. The amount of dust used on each premises varied considerably since none of the farms were of the same size. However, two pounds of DDT dust per farm was a fair average.

A condensation of available data is given in table II.

A comparison of mite and lice indices from rats taken on treated farms shows that 10% DDT applied to rat runs did not reduce the mite or lice populations below those normally found on the domestic rat.

Xenopsylla cheopis indices obtained from rats trapped on farms where 10% DDT had been applied to rat runs showed a definite reduction of this species of flea. However, two applications of 10% DDT dust to rat runs may be necessary to effect adequate control when an unusually high infestation is encountered.

Table II

Comparison of Ectoparasite Indices on Farms Dusted with 10% DDT and Undusted Farms in Rural Areas

TABLE II

Comparison of Ectoparasite Indices on Farms Dusted with 10% DDT and Undusted Farms in Rural Areas

	April							May						
	Trapping Period	Number of rats caught	*X. cheopis* index	*L. segnis* index	*E. gallinacea* index	*L. bacoti* index	*P. spinulosa* index	Trapping Period	Number of rats caught	*X. cheopis* index	*L. segnis* index	*E. gallinacea* index	*L. bacoti* index	*P. spinulosa* index
Ectoparasite indices on treated farms	4/23–5/3	29	0	0	2.2	4.4	4.1	5/21–5/29	37	0.4	0.2	7.7	4.5	4.9
Ectoparasite indices on check farms	4/23–5/14	9	3.1	4.0	5.3	9.9	5.9	5/16–5/29	10	7.3	2.8	2.8	1.9	2.6

	June							July						
	Trapping Period	Number of rats caught	*X. cheopis* index	*L. segnis* index	*E. gallinacea* index	*L. bacoti* index	*P. spinulosa* index	Trapping Period	Number of rats caught	*X. cheopis* index	*L. segnis* index	*E. gallinacea* index	*L. bacoti* index	*P. spinulosa* index
Ectoparasite indices on treated farms	6/18–6/28	33	2.0	0	1.6	8.7	3.1	7/23–8/2	27	2.5	0.1	0.4	0.3	1.6
Ectoparasite indices on check farms	6/11–6/28	11	9.8	0.1	4.1	5.7	5.3	7/23–8/2	11	5.3	0	0	0.4	1.4

	August							September						
	Trapping Period	Number of rats caught	*X. cheopis* index	*L. segnis* index	*E. gallinacea* index	*L. bacoti* index	*P. spinulosa* index	Trapping Period	Number of rats caught	*X. cheopis* index	*L. segnis* index	*E. gallinacea* index	*L. bacoti* index	*P. spinulosa* index
Ectoparasite indices on treated farms	8/21–8/30	10	0.2	0	1.2	0	0.6	9/19–9/20	14	0.07	0	0.2	1.4	0
Ectoparasite indices on check farms	8/20–8/30	4	2.7	0	0	1.7	8.2	9/17–9/20	6	2.5	0.2	0	2.2	4.3

No significant reduction in the numbers of *L. segnis* could be descerned. This may have been due to the low indices recorded for this species.

Larger numbers of *E. gallinacea* were found on rural rats than on rats trapped in urban areas. A comparison of the sticktight flea indices from rats taken on untreated farms and farms treated with 10% DDT dust shows no significant reduction in numbers of *E. gallinacea* on rats from treated farms. It is also evident that the sticktight flea distribution is irregular.

Summary

It was determined that a high percentage of rats was infested with fleas, lice, and mites throughout the year in Lavaca County, Texas. The highest incidence of the oriental rat flea, *Xenopsylla cheopis*, was during June, July, and August. The mouse flea, *Leptopsylla segnis*, was most abundant during March and April.

Houses and business establishments in Shiner and Moulton in Lavaca County were dusted with 10% DDT in pyrophyllite. Approximately one-half pound of the dust was applied to small cafes and residences and two to eight pounds for large establishments such as cotton gins and warehouses. Hallettsville was left undusted as a check town. Indices of *Xenopsylla cheopis* and *Leptopsylla segnis* were significantly reduced in the dusted towns.

DDT studies were also carried on in rural areas since at least half of the endemic typhus cases occurring in Lavaca County have been in the rural areas. Twenty-four farms were used in the studies. Six farms were undusted checks and eighteen were dusted. An average of two pounds of DDT dust per farm was used. *Xenopsylla cheopis* showed a definite reduction on the treated farms. No significant reduction in the numbers of *Leptopsylla segnis* could be discerned. This may have been due to the low indices recorded for this species. No significant control of *Echidnophaga gallinacea* was noted on treated farms.

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INCIDENCE OF LEPTOSPIROSIS IN MAN AND RODENTS IN GALVESTON

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Up to 1935 only twelve authentic human cases of Weil's disease were reported from the United States and Canada. In 1938 Packchanian reported several additional cases of Weil's disease from New York, Massachusetts, Pennsylvania, Maryland, Ohio, Michigan, Missouri, and Kansas. Three years later, the same author reported 40 human cases of Weil's disease from the United States diagnosed by agglutination test. Since 1938, the agglutination test has been popularized and, as a result, several dozens of additional human cases of Weil's disease have been diagnosed during the past eight years from various states. However, no authentic human cases have been reported in Texas prior to this communication.

Due to newer laboratory methods, the differential diagnosis of Weil's disease from infectious hepatitis and yellow fever has become relatively easy. As clinicians and laboratory workers become more familiar with Weil's disease, less typical and even sub-clinical cases are gradually unfolding themselves.

That wild rats *(Rattus norvegicus and R. rattus)* are the chief reservoir of *Leptospira* is well known. The percentage of rats naturally infected with *Leptospira* varies from location to location, and to a certain extent with the methods of the investigators. On an average, about 20 to 25 per cent of wild rats *(R. norvegicus* and *R. rattus)* throughout the world are possibly carriers of *Leptospira.*

The object of the present communication is to give information concerning the incidence of rats *(R. norvegicus)* naturally infected with *Leptospira*, and to report briefly on five human

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cases of Weil's disease diagnosed in Galveston, Texas, for the first time, by agglutination and animal inoculation tests.

Method and Material

Source of Wild Rats and Method of Examination. Wild rats *(R. norvegicus)*, which had been caught in "dead traps" in various sections of Galveston, were delivered to the laboratory within four to six hours after the animal's death. The kidneys of the dead rats were excised. Some of the tissues from the pelvis of the kidneys was macerated in saline in mortar; the suspension was filtered through gauze, and examined microscopically by darkfield illumination for presence of motile *Leptospira*. All specimens positive for spirochetes were inoculated into young guinea pigs. The ear blood of these guinea pigs was examined microscopically daily from the sixth to the twelfth day after inoculation for the evidence of *Leptospira*.

When a patient was suspected of having Weil's disease, about 10 cc. of blood was collected aseptically, defibrinated, and then one or two cc. blood was inoculated intraperitoneally into each of two guinea pigs and two deer mice *(Peromyscus maniculatus)*.

Aggultination Test. Samples of serum from suspected human cases and from some rats were tested for presence of agglutinins with Type I *Leptospira icterohaemorrhagiae* by the Schüffner microscopic agglutination test.

Culture Test. The heart blood of guinea pigs experimentally infected with the kidneys of wild rats or with the blood of suspected patients, was inoculated in liquid and semi-solid media. The tubes were rubber-capped, and incubated two to four weeks at 30° C., then examined microscopically for growth of *Leptospira*.

Experimental Data

The results of these studies pertaining to five human cases are summarized on Table I.

TABLE I

Five Human Cases of Weil's Disease in Galveston, Texas Diagnosed by Agglutination and Animal Inoculation Tests.

Case No.	Color	Sex	Age	Attending Physician	Occupation	Onset of Illness	No. days after onset specimen collected	Serum dilution & agglutination reaction						Animal Inoculation Tests	Outcome of Illness
								1:100	1:300	1:1,000	1:3,000	1:10,000	1:30,000		
1	C	M	30	Dr. H. B. Griffin Dr. G. Herrmann Dr. T. G. Price	Unemployed	May 31 1946	16	+++	++++	++++	+++	+++	++	+	Recovered
2	W	M	50	Dr. Oliver Lewis Dr. W. W. Nesbit	Merchant Seaman	Sept. 21 1946	29	++	++++	++++	++++	++++	+++	---	Recovered
3	C	M	39	Dr. Lloyd Gregory Dr. Raymond Gregory	Unemployed	July 8 1947	6 11	++ +++	++ +++	— +++	— +++	— —	— —	—	Died
4	C	M	49	Dr. G. Herrmann Dr. T. G. Price Dr. J. A. Rider	Farmer	Nov. 8 1947	11	++	+++	++	+	—	—	—	Recovered
5	W	M	10	Dr. Lloyd Gregory Dr. A. E. Hansen	Student	Feb. 14 1948	18	++	+++	+++	+++	++	—	+	Recovered

The serum samples from all cases agglutinated with Type I *Leptospira icterohaemorrhagiae* in dilution from 1:1000 to over 1:30,000. The agglutination reaction of these samples was prompt and clear cut within four to six hours, and diagnostic for Weil's disease.

In serum samples collected from patient #3 in the early stages of the disease, the agglutination titer was low. Other samples collected five days later showed a rise in the agglutination titer from 1:100 to 1:1000, indicating current infection. This patient died 11 days after the onset of the illness.

Leptospira was isolated from the urine of patient #1, and from the blood of patient #5, by animal inoculation tests.

Patient #5, a 10-year-old student who was bitten by a wild rat and who subsequently killed the rat, was admitted to the hospital for rat-bite fever. He showed no symptoms for Weil's disease except nausea and vomiting a few days after rat bite. Specimen of blood from this patient was inoculated into a guinea pig and a mouse *(Mus musculus)*. The guinea pig died within nine days, with characteristic jaundice and hemorrhage. *Leptospira* was demonstrated in the blood of that guinea pig and was cultured *in vitro* in liquid and semisolid media. The mouse *(Mus musculus)* which was inoculated with this patient's blood showed a few *Leptospira* in its circulating blood only on the eighth day, and later apparently became a chronic carrier of *Leptospira*. The *Spirochaeta morsus muris* was never demonstrated in the blood of this mouse in spite of frequent microscopic examinations for about three months. Deer mice *(Peromyscus californicus* and *P. maniculatus)* inoculated with this strain of *Leptospira* contracted acute infections and died before the sixth day; two days before death the blood of these deer mice was swarming with *Leptospira*.

In the kidneys of 21 out of 93 rats *(R. norvegicus)* examined, active *Leptospira* was demonstrated by direct microscopic search with darkfield illumination.

Agglutination tests were negative on serum samples from 18 wild rats *(R. norvegicus)*. No *Leptospira* were observed in the kidneys of these rats. However, the serum samples of a wild rat *(R. norvegicus)*, whose kidneys were positive for motile *Leptospira*, gave agglutination reaction with Type I

Leptospira icterohaemorrhagiae in dilutions up to 1:30,000, and the serum sample from an experimentally infected mouse *(Mus musculus)*, whose kidneys also were positive for *Leptospira*, gave agglutination reaction in dilutions up to 1:10,000.

Out of 23 guinea pigs inoculated with the kidneys of 14 rats, 21 showed actively motile *Leptospira* by microscopic examination, but recovered and survived; the other two guinea pigs died of icterohaemorrhagic spirochetosis.

Three laboratory-reared albino rats *(R. norvegicus)* inoculated with the kidney suspension of dead wild rats positive for *Leptospira*, became chronic carriers. One and two months later, these rats were sacrificed and *Leptospira* was demonstrated microscopically in their kidneys. The kidneys of these rats, while fresh, were macerated and the suspension inoculated into young guinea pigs and deer mice. All the test animals contracted fatal leptospirosis and died with characteristic jaundice and hemorrhage.

Four laboratory-reared white mice *(Mus musculus)*, inoculated with *Leptospira* derived directly from wild rats, also became carriers of *Leptospira*. When these animals were killed 38, 40, 50, and 69 days later, active *Leptospira* was found in the kidneys of each animal. The mice *(Mus musculus)* and rats *(R. norvegicus)* inoculated with the same kidney suspension from these mice became chronic carriers of *Leptospira*, while guinea pigs and deer mice *(Peromyscus)*, inoculated with the same material, died of leptospirosis.

Discussion

Typical Weil's disease is characterized by malaise, initial chill, sudden onset of high fever, nausea, vomiting, severe muscular pain, prostration, epistaxis, hempotysis and jaundice. The disease in men, however, may present various degrees of severity. About two to five per cent of the cases are probably fatal. However, most of the cases are mild and self-limited. Subclinical cases without jaundice and muscular pains are not uncommon.

Since antibodies of *Leptospira* remain for at least several years in the patient's circulating blood, retrospective diagnosis of Weil's disease may be performed when necessary or desirable.

Inasmuch as less virulent strains of *Leptospira* may fail to produce death in guinea pigs, but may produce antibodies such agglutinins and lysins as a result of subclinical infection, the serum of these test animals should be subjected to serological tests before and after a two-week period of inoculation, in order to detect subclinical Weil's disease or less virulent strains of *Leptospira*. It must be kept in mind that there are several serological strains of *Leptospira*, and that a negative agglutination reaction with one type or strain of *Leptospira* will not eliminate the possibility of Weil's disease.

It is noteworthy that when *Leptospira* is microscopically demonstrated in the kidneys of wild rats several hours after their death, and when the kidneys of these animals are macerated and inoculated into susceptible test animals such as guinea pigs, the test animals may fail to succumb to the disease. This is probably due to less virulent or post-mortem attenuation of *Leptospira*. When spirochete is passed into normal rats *(R. norvegicus)*, the animal contracted abortive blood infection and became carrier of the microorganism, and such animals later sacrificed and the kidneys containing active *Leptospira* macerated within one hour and inoculated into guinea pigs, the latter almost invariably succumbed to leptospirosis.

Apparently the wild rats *(R. norvegicus)* and mice *(Mus musculus)*, when naturally or experimentally infected with *Leptospira*, will not only become carriers, but may also produce agglutinins. These antibodies apparently keep spirochetes out of the circulating blood; but the *Leptospira* will flourish in the kidney pelvis of the animals, and are periodically eliminated in the urine.

It is a definite fact that regular house or laboratory mice *(Mus musculus)* react to *Leptospira* in the same way as rats *(R. norvegicus)*. In contrast, certain deer mice, such as *Peromyscus maniculatus gambelii*, *P. eremicus*, and *P. californicus*, have extreme susceptibility to *Leptospira* and die of leptospirosis within six to ten days. These deer mice are the only animals known whose blood will be swarming with *Leptospira* at the latter part of the disease.

During 1937, in New York, a dog killed a rat, and became infected with leptospirosis (unpublished data of one of the writers, A.P.). Similarly, children are known to contract Weil's disease while playing with infected dogs.

Weil's disease may be looked on as an occupational disease. The data available on many cases indicates the majority of the patients lived or worked in places where wild rats might have been present and where working conditions were conducive to skin abrasions. Persons who have skin abrasions and have been in contact with wild rats, directly or indirectly and who become ill, should be examined in order to confirm or eliminate the possibility of Weil's disease. Since antibodies are present in the circulating blood of the patient for many years after recovery from the disease, retrospective diagnosis of this disease can be performed when necessary or desirable.

Summary

1. Five human cases of Weil's disease have been diagnosed for the first time in Galveston, Texas. One of these cases terminated fatally, another was subclinical, and the remaining three were typical clinical cases.

2. Out of 93 wild rats *(R. norvegicus)* collected in Galveston, Texas, 21 were found, by direct microscopic examination of the kidneys, to be carriers of *Leptospira.*

3. Regular house or laboratory mice *(Mus musculus)*, inoculated experimentally with *Leptospira* became chronic carriers, like *R. norgevicus.* On the other hand, deer mice *(Peromyscus maniculatus gambelii, P. californicus)*, inoculated with material containing *Leptospira*, contracted the infection and died of icterohaemorrhagic spirochetosis within six to ten days.

4. Rats *(R. norvegicus)* and mice *(M. musculus)* which gave positive microscopic findings for *Leptospira* in the kidneys, showed also the presence of agglutinin for *Leptospira* in their circulating blood.

5. It is concluded that Weil's disease is more prevalent than is usually suspected, and that with the increased vigilance of clinicians and laboratory workers, many more cases will be revealed.

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PENETRATION OF AN EXPERIMENTAL SARCOMA BY NERVE FIBERS FROM THE SPINAL CORD

DONALD DUNCAN AND NICHOLAS J. BELLEGIE*

During the course of a study on spinal cord compression produced by implantation of tumors in rats, a very clear-cut example of invasion of tumor by nerve fibers was encountered. As this observation is of significance both from the standpoint of the relation of nerve fibers to malignant growths and to the regenerative powers of the spinal cord, the entire series was carefully examined with the results and conclusions that follow.

A brief but comprehensive account of work on the role of nerves in cancer may be found in Behan's text, 1938. Most other current texts on pathology and neoplasia pay little or no attention to the work that has been done on the innervation of tumors. Ewing (1928) merely states that the formation of nerve fibers in sarcomas has not been demonstrated. He gives no references. Karstner (1942) mentions the subject and refers the reader to Ryrie (1933) for information and the authors of a number of other books examined say nothing. This is scant attention to the considerable work on the subject and no doubt reflects the prevailing attitude that innervation is not an important aspect of tumor growth and development. While it is probably true that innervation plays an insignificant role in the growth and spread of malignant neoplasms this is not due to an inability to demonstrate nerve fibers in tumors. In fact, with but one exception (Meyer, 1910) all who have studied this problem agree that nerve fibers can be found in both benign and malignant growths. On the other hand the various reports differ widely on the extent, ultimate termination and significance of the nerve fibers encountered.

History of the study of innervation of tumors starts with the observations of Hugh Young in 1897 and apparently ends with a paper by Oertel in 1937. At least, no subsequent references have been located. Using the methylene blue technique

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of Ehrlich, Young was able to demonstrate nerve fibers in five of ten human tumors of non-nervous origin. Some of the fibers he found were deep within sarcomas. He believed that nerves were as much a part of the structure of a sarcoma as the blood vessels and he suggested a vasomotor function for them. A direct contact between nerve fibers and tumor cells was neither established nor claimed by Young. The next paper on the presence of nerves in neoplasms was by K. Meyer in 1910. With the Bielschowsky method he was unable to find fibers in spontaneous tumors.

In subsequent years the presence of nerves in tumors was generally affirmed but with widely divergent results as to detail and diametric opinions as to their significance. Goldmann (1911) noted the widespread occurrence of myelinated fibers in carcinomas and sarcomas stained by the Weigert method. Itchikawa with various associates (see Itchikawa and Takahata, 1928) repeatedly maintained that nerve fibers are found in experimental and spontaneous tumors, that they grow with the tumor cells, and constitute an integral part of the phenomena of neoplasia. The same results and opinions were expressed by Oertel in a series of papers between 1928 and 1937. Martynow (1930), using a modification of the Golgi method, found nerve fibers in experimental tumors of rats and in the epithelium of skin carcinomas.

In contrast to the above mentioned, Herzog (1928) in an extensive study of 100 human tumors found nerve fibers limited to the stroma of uncircumscribed tumors. In no instance did he find nerve fibers in contact with the tumor cells. He concluded that tumors are devoid of specific nervous influence although the possibility of vasomotor influence exists. Herzog found support for his findings and conclusions in the work of Nakomoto (1926) who failed to demonstrate nerve fibers in malignant growths, and also in the experiments of Milone. Milone (1929) placed rat sarcoma in contact with the central end of the cut sciatic nerves of this animal. Although nerve fibers grew into the tumor they had no relation to the tumor cells and failed to develop any specific endings. No effect on the development of the neoplasm was noted in comparison to growth in other sites. Hunda and

Kura (1928), likewise, were unable to find other than occasional nerve fibers in the stroma of tumors.

The findings and conclusions of Herzog were subsequently confirmed and upheld by Ludford (1930) and by Ryrie, and their views are subscribed to by Willis (1948). On the other hand, Athias and Dias (1932) are in accord with Oertel in most respects. They agree with him on the abundance and constant occurrence of nerve fibers within all types of tumors. Athias and Dias used the Cajal technique and studied an extensive series of experimental and spontaneous tumors. They saw no evidence of nerve endings within tumor cells as claimed by Oertel but did find nerve fibers in all types of tumor examined and they concluded that such fibers are a constant feature of both parenchyma and stroma. They cite 58 papers on the subject of nerves in tumors and anyone interested in the complete story of this subject is referred to their paper.

Oertel restated his position without additional direct evidence in 1937 and from that date until the present time no papers on innervation of tumors have been listed in the Cumulative Index.

Concerning the other phase of the problem, namely, growth of severed fibers in the central nervous system, it is generally believed that such growth is either non-existent or exceedingly limited. Except for the unconfirmed report of Sugar and Gerard (1940) on effective regeneration of the spinal cord in rats, all other experiments on mammals lead to one conclusion; namely, that any regenerative growth is exceedingly limited and completely ineffectual. The most recent attempts are those of Brown and McCouch (1947) who found a few fibers in scar that might have come from the severed ends of the spinal cord. Some years ago, LeGros Clark (1942) inserted a variety of substances in the brain in an attempt to induce their invasion by fibers of central origin but without success.

Materials and Methods

The material used in this study was obtained from a series of rats subjected to implantation of Walker rat sarcoma No. 319 into the vertebral canal. In one group the implantation

was intended to be epidural and probably was except in one instance as will be mentioned in the observations. In another series tumor implantation followed partial transection of the spinal cord which was done a few days before implantation. In this series the integrity of the dura was necessarily destroyed.

All observations of nerve fibers containing tumor were made in cross sections stained by Bodian's protargol method as modified by Drager (1947). Slides studied were from rats sacrificed 24–72 hours after paralysis induced by compression of the implanted tumors, and also from animals that had recovered following surgical removal of the tumor. In all, sections from fifteen paralyzed rats and eleven recovered rats were studied. Of these, nerve fibers were found among the tumor cells in two of the paralyzed rats and in three of the recovered ones.

Observations

Nerve fibers within the tumor were seen in only one of the epidural transplants. In this animal a gap in the dura brought the tumor cells in direct contact with the pia and nervous tissue of the cord, see Figure 1. In all the others of the epidural series the dura formed an intact barrier between the sarcoma and the spinal cord and no nerve fibers within tumor were seen. Although this one instance of lack of integrity of the dura may have been due to invasion and destruction of the membrane by the sarcoma, both the appearance of the sections in the one animal presenting this feature and the complete lack of any signs of penetration in the other animals make it fairly certain that the dura was inadvertently opened at the time of implantation. Nerve fibers were found among

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PLATE I

FIG. 1. A low power view of the vertebral canal of a rat containing nerve infiltrated sarcoma, S, and compressed spinal cord, C. In this section dura is absent between points indicated by X. Arrows indicate line of contact between tumor and spinal cord, and a dot marks the point of maximum penetration of the sarcoma by nerve fibers. Protargol stain, X 26.

FIG. 2. A high power view of a portion of the tumor shown in Figure 1. Several of the nerve fibers in the picture are indicated by arrows. Note differences in caliber, branching, and close relation to tumor nuclei. Protargol stain, X 1100.

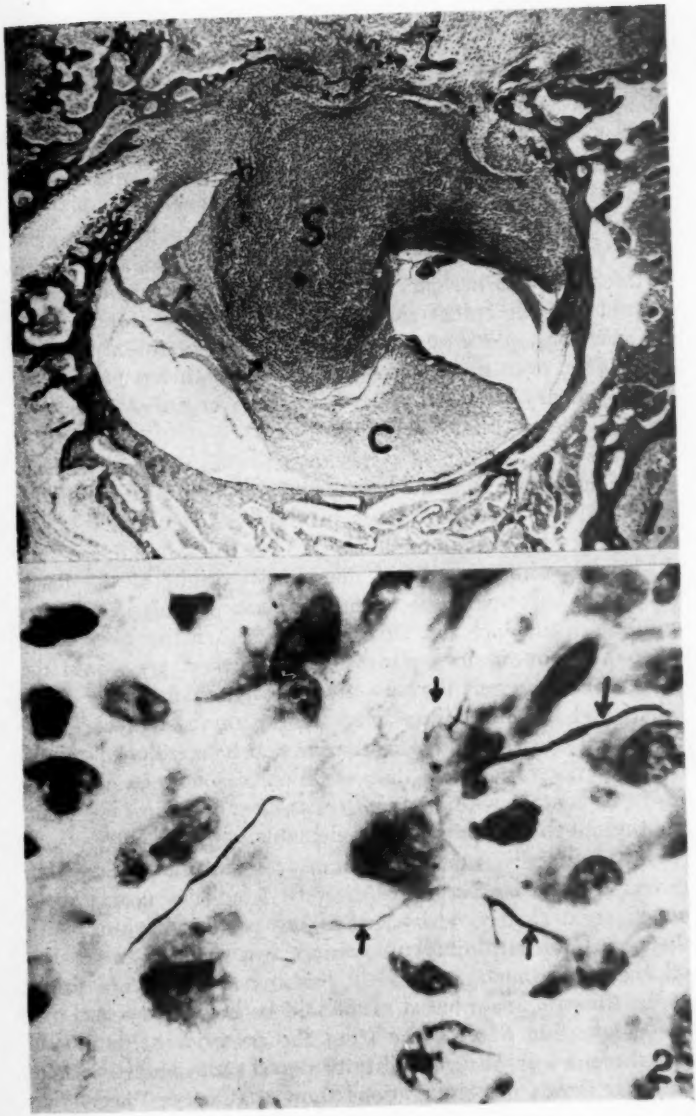

PLATE I

the tumor cells in all four animals subjected to partial transection of the cord prior to tumor implantation. It is to be noted that in each of these the dura had been rather widely opened prior to tumor implantation.

In the positive cases nerve fibers are abundant in the zone of immediate contact between cord and tumor and rapidly diminish in number towards the central mass of the neoplasm. Occasional fibers can be found 500 micra within the tumor but no deeper. Most of them occur in a zone 200 micra in thickness and here they are really numerous only within 50 micra of the spinal cord. Long and careful search of the extravertebral portions of the tumor failed to reveal a single nerve fiber even though numerous small branches of the posterior primary divisions must have been engulfed or pushed aside by the sarcoma.

In the one instance of tumor penetration without previous operation on the spinal cord the histological picture clearly indicates that the nerve fibers penetrated the tumor and not vice versa. Likewise, the sections from this animal are conclusive in ruling out direct dorsal root origin of the fibers within the sarcoma. The other four examples of nerve fibers within sarcoma are not clear as to whether the fibers were surrounded by tumor cells or whether they grew into the neoplasm. However, they do strongly suggest the latter possibility, and this in conjunction with the unquestionable penetration of tumor by nerve in the previously mentioned animal leads to the conclusion that in all five examples of nerve fibers in contact with sarcoma cells, the nerve fibers and not the tumor cells are the invasive elements.

In the partially transected animals some of the fibers within tumor can be seen coming directly from the dorsal roots, others seem clearly derived from the posterior columns. In the nontransected animal no contact was made between dorsal roots and the tumor and in this instance the cord origin of the fibers is unequivocal. It should be noted, however, that the penetrating fibers come from the posterior columns and are, therefore, in all probability of dorsal root ganglion origin. In other words the observations show that nerve fibers lying within the spinal cord and surrounded by neuroglia rather

than neurilemma have some power of regeneration. The findings neither demonstrate nor deny that fibers arising from cell bodies within the spinal cord may exhibit regenerative and invasive powers.

Within the tumor the fibers vary in size from fine to moderately coarse ones. Beyond noting that there seems to be no one size category involved, no significance is attached to the caliber of the penetrating fibers. Likewise, the usual diversity of shapes and branches seen whenever nerve fibers are penetrating a new environment may be found among the sarcoma cells. True end-bulbs are not present while numerous examples of fine tendrils are, this probably indicates that active and non-obstructed growth was taking place up to the time of sacrifice. As shown in Figure 2 the relation of the nerve fibers and the tumor cells is intimate to the point of suggesting actual penetration of the malignant cells by nerve fibrils. No doubt similar finds have been interpreted as evidence of cell penetration even though neither the sectioning nor the staining of this or similar material is sufficiently critical to be of any value in drawing such a conclusion.

Comment

The present observations are one more example of the penetration of malignant tumors by nerve fibers. Due to their abundance, clarity, and the fact that they are displayed by the Bodian technique any lingering doubts based on previous reports of this phenomenon should be dispelled. On the other hand the observed absence of nerve fibers in extravertebral portions of these tumors in addition to the peculiar conditions of pressure and intimate contact between spinal cord and tumor associated with the phenomenon of nerve invasion of neoplasm give support to those who claim that nerve fibers in tumors are neither abundant nor universal. These and previous studies would seem to justify the opinion that the physical interrelationship between neoplasms and nerve fibers should at least be kept in mind until more is known about each of them; and further, that the very extensive studies on the subject should not be dismissed entirely in any comprehensive work on the subject of cancer.

With respect to the source of the penetrating fibers there can be no question of the fact that they come from the spinal cord in one instance. However, as mentioned previously, these fibers coming as they do from the posterior white columns must be for the most part derived from the dorsal root ganglion cells. Since even these ordinarily fail to show appreciable regenerative capacity once they are within the cord, the present observations reinforce the opinion that this failure is due to lack of a suitable environment for continued growth and not to an inherent lack of capacity.

Summary and Conclusion

Invasion of Walker rat sarcoma No. 319 by nerve fibers from the posterior white columns of the spinal cord, and directly from the dorsal roots is described.

The finding demonstrates again the possibility of the invasion of malignant growths by nerve fibers, and furnishes additional evidence in support of the belief that one of the causes of lack of regeneration in the central nervous system is the absence of a suitable substrate for advancing axons.

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A NOTE ON THE PROPER TECHNIQUE OF PRECORDIAL ELECTROCARDIOGRAPHY

EDMOND K. DOAK*

Ziegler (1948) has recently reported the source of an important error in recording the precordial electrocardiograms of infants and children. He found that the application of electrode paste conjointly to all the precordial electrocardiographic positions, establishing electrical contact through the layer of paste between these positions, allowed a transmission of electric charges so that precordial electrocardiograms made following this type of skin preparation were definitely different from the records secured on the same patient when only one area on the skin was prepared at a time.

Ziegler stated that the technical error "is not as easily made in adults in whom the distance between the positions for the precordial leads is greater than in infants and in small children." An electrocardiographic study of 10 adult individuals has been made in an effort to evaluate this opinion. In each individual a complete electrocardiographic study was made, recording the three standard leads, the three augmented unipolar extremity leads and the precordial leads V_3R, V_1, V_2, V_3, V_4, V_5, and V_6. All seven precordial positions were prepared conjointly with a liberal amount of electrode paste for the first set of precordial tracings. The result was a liberal and continuous smear of electrode paste from the left axilla to the mid-portion of the right anterior chest wall. After electrocardiograms had been secured from each of these positions in turn, the entire chest was cleansed with warm water and dried thoroughly with a towel. A second set of precordial electrocardiograms was then obtained by preparing each position separately, obtaining the tracing from this area and then cleansing thoroughly. The various positions were marked with an indelible skin pencil so that variation in the placement of the precordial electrode in the two sets of electrocardiograms

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was minimized. In most instances the small indentation resulting from previous pressure of the electrode permitted the technician to replace it with a minimum of error. The subject of the test remained supine throughout the entire procedure.

It was found that in some subjects there was no significant difference between the two sets of precordial electrocardiograms obtained by following the two techniques of skin preparation. In other subjects however the differences were held to be significant and of a type that might cause clinical confusion in certain cases.

In no subject with a wide thorax was there any significant difference in the two sets of tracings. In one subject whose broad chest was covered by an extraordinary amount of hair the excessive amount of electrode paste held on the skin by this hirsuties did not cause a significant variation.

Five subjects or one half the group studied had chests regarded as being narrower than average. A well developed slender young man with a heavy growth of hair across the anterior chest wall showed significant differences between the two sets of tracings, as did two slender but well nourished young women. Another young woman of essentially the same habitus demonstrated a minor difference in lead V_3 only. An elderly man with chronic heart disease having a very narrow chest and a rather heavy growth of hair upon it showed no significant difference in the two sets of electrocardiograms. This subject, alone of the group studied, had a definitely increased anterior posterior diameter of his chest, and it may well be that separation of the chest wall from the heart could account for his failure to demonstrate those changes which were more usual in others of his general habitus.

If we assume that precordial electrocardiograms secured by separate preparation of the respective positions are most nearly accurate, we may then assume that any variation in the form of these tracings obtained after conjoint preparation is a technical error. All these errors, whether deemed significant or negligible, have been found to be of the same general type. The errors are most apt to be evident over the left precordium in the region of the interventricular septum and are least evident in the left axilla and over the right chest

where the leads are taken farthest from one another. Variations from the baseline, whether positive or negative, are carried through the layer of electrode paste from one position to adjacent positions but the magnitude of these potential changes is not significantly lessened at its source by this dissemination. That is to say, a low R wave may gain in height by conduction from the adjacent position but height of the R wave in this adjacent position is not lessened materially by conduction of the charge to the surrounding area.

Figure 1 illustrates the effect of improper preparation on the Q R S complexes of the precordial electrocardiogram of a slender young woman. Assuming that the lower set of tracings is accurate, one may see in the upper set of tracings, obtained

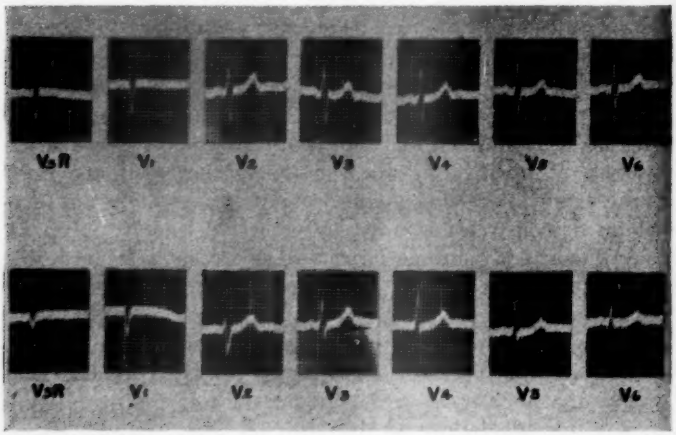

FIG. 1. Two sets of precordial electrocardiographic tracings made on a slender but well-nourished young woman. The upper set of tracings was made after conjoint skin preparation of all the precordial positions while the lower set of tracings was made following separate preparation of each position. The effect of improper skin preparation, seen in the upper set of tracings, is discussed in the text.

after conjoint preparation, that in V_5R the deep S wave is due more to surface conduction than to internal negative potential but that V_1 has lost none of its negativity by diffusion. The S wave which is evident in V_1 and V_2 of the lower tracings has been spread in the upper tracing across the entire

precordium, while the R wave of V_3 has been conducted to the 2 position. There are no apparent significant variations in the T waves of the two sets of tracings. In the lower set of tracings one might infer with some accuracy that lead V_2 lay over the right ventricle while V_3 lay over the left ventricle. In the upper set of tracings no such conclusion could be drawn, for leads V_2, V_3, V_4, V_5, and V_6 all show essentially the same characteristics.

In Figure 2 the same type of error may be seen. Leads V_3R, V_3, and V_5 show the most significant variation from the accurate tracing in the lower portion of the figure. Other errors

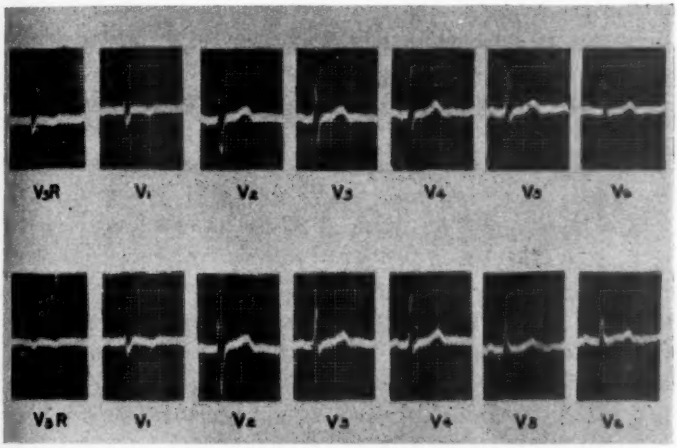

FIG. 2. Two sets of precordial electrocardiographic tracings made on a slender but well-nourished young woman. The upper set of tracings was made after conjoint skin preparation of all the precordial positions while the lower set of tracings was made following separate preparation of each position. The effect of improper skin preparation, seen in the upper set of tracings, is discussed in the text.

noted in this study are of the same general type as those illustrated in these two figures and are similar to those errors demonstrated by Ziegler (1948). Such errors, demonstrated here in electrocardiograms made on normal individuals, could be magnified in abnormal situations and could nullify much of the precise information obtainable from precordial electrocardiography. This source of error should be kept in mind

when considering the use of any of the various devices recommended for holding one or more exploring electrodes on the anterior chest wall.

Summary

Application of electrode paste to all the precordial positions conjointly rather than separately may lead to the introduction of important errors in the electrocardiograms so obtained. These errors are most likely to be found in electrocardiograms made on individuals with narrow chests.

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THE NUTRITIONAL SIGNIFICANCE OF METHIONINE AS ILLUSTRATED IN POULTRY

MELVILLE SAHYUN*

Poultry and egg consumption in the U.S.A. has for the past several years been steadily gaining momentum. Statistical studies, from 1909 to 1940, indicate an average yearly consumption of about 19 pounds of chicken and 310 eggs per capita (Booher). Since 1940, the demand on these two important commodities has been considerably increased that it is now estimated at about 27 pounds of chicken and 350 eggs per capita per year. Considering the population of this country to be 140 million, we must then consume some 3,830,000,000 pounds of chicken and 49,000,000,000 eggs (Kraybill).

According to Chatfield and Adams the live weight of a broiler, age 8 to 12 weeks, ranges from 1½ to 2½ pounds, of a fryer, age 14 to 19 weeks, from 2½ to 3½ pounds, and of a roaster, age 5 to 9 months, from 3½ pounds and over. The edible portion of the flesh of birds in each category, including as edible portion the flesh, fat, skin, and giblets, contains approximately 20 per cent protein, except in case of very fat birds.

In order to produce this tremendous amount of chicken and eggs, obviously huge quantities of feed are required and must be produced. The data presented in Table I, show the relative quantities of feed required to produce one pound of live chicken of each category and one dozen eggs. Assuming therefore that about 4 pounds of feed can on an average be converted by the chicken to one pound of meat, and 5 pounds of feed are required by the laying hen to produce one dozen eggs, then the amount of feed necessary is 15,320,000,000 and 20,400,000,000 pounds, respectively. These figures do not take into consideration the yearly requirement of the maintenance

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of the laying hen. This may be estimated at 1,500,000,000 pounds of feed. In brief, a total of more than 37 billion pounds of feed must be raised for the production of chickens and eggs we annually consume.

TABLE I

Average Feed Consumption of Chickens

Chicken	Age Weeks	Weight lbs.	Feed consumed Per lb. Live Weight lbs.	Efficiency conversion Factor Per cent
Broiler	9	1.85	3.6	27.8
Broiler	12	2.57	3.64	27.5
Fryer	14–17	3.52	4.80	20.8
Roaster	20 and over	4.17	5.17	19.3

Average Yearly Feed Consumption of White Leghorn Pullets for Maintenance and for Eggs

Laying Pullet producing Per year Eggs	Feed Consumed for Maintenance lbs.	Eggs lbs.	Total lbs.	Feed Consumed Per Dozen Eggs lbs.
80	64	7.14	71.40	10.67
120	64	10.69	74.69	7.47
160	64	14.24	78.21	5.87
200	64	17.81	81.81	4.91

The chicken requires a more complicated and exacting mixture of nutrients than most domestic animals. It is particularly sensitive to the lack of a number of dietary factors such as vitamin K and choline, manganese, potassium, calcium, and magnesium (Almquist). The chicken also requires a large percentage of protein in its diet.

The raising of poultry is a big industry and poultrymen raise fowls for meat, eggs, and breeding purposes. For production of chicken meat, we distinguish three categories: broilers, fryers, and roasters. From the data presented in Table I, we note that as chickens become older, their ability

to convert feed into meat is greatly reduced. It is then costlier to feed a roaster than a fryer or a broiler.

The laying hen is a class by itself. It requires food for maintenance and for egg production. A four-pound hen may in one year lay more than 25 pounds of eggs (Fritz). It is evident that this egg production adds materially to the feed requirement of that hen. As to hatchability, the nutritional requirement of the laying hen becomes even more exacting. These facts are well recognized by poultrymen, but are not as obvious to laymen and to those who are not versed in this field.

In connection with nutritional requirements of poultry, Ringrose stated: "The greatest concern of the nutritionist and the feed manufacturer is with the conditions resulting from partial dietary deficiencies. Such deficiencies do not become serious enough to present an easily recognized syndrome, yet normal results are not obtained. When such a situation is suspected under farm feeding conditions, a satisfactory solution of the problem calls for intelligent co-operation of all concerned. The answer, frequently lies in the variability in the nutritive value of feedstuffs combined with the particular set of conditions on the farm.

Amino Acids Requirement of the Chick. The basic components of poultry nutrients are not qualitatively different from those of other domestic animals or humans. They are water, essential mineral salts, vitamins and growth-factors, proteins, fats, and carbohydrates. Quantitatively however, the composition of the chicken's diet differs in several respects from that of the white rat or of man. In Table II, we present the composition of a typical synthetic diet which has been shown by Grau to be satisfactory for the normal growth of the chick. It is evident from this table that the chick requires a large assortment of amino acids, mineral salts and vitamins. In Table III, we show the essential amino acids requirements of the chicken, the rat, and man. Here again we note that the chick needs a larger number of different essential amino

TABLE II

Experimental Synthetic Diet for White Leghorn Chicks, Previously Reared on a Commercial Type of Diet until they were 2 weeks old. This Table is according to Grau.
Amounts are expressed in grams per 100 grams Mixture.

Amino Acids	Amount grams	Vitamins and other Ingredients Mineral Mixture	Amount grams		Amount grams
Dl-Alanine	1.5	Sodium Bicarbonate	1.5	Soy bean oil	5.00
L-Arginine. HCl	1.2	Calcium gluconate	8.0	Sardine oil*	0.25
L-Cystine	0.5	Tricalcium phosphate	2.0	Tocopherols	0.05
L-Glutamic Acid	5.0	Dipotassium phosphate	0.5	Choline. HCl	0.2
Glycine	1.8	Potassium chloride	0.3	Inositol	0.1
L-Histidine. HCl	0.8	Manganese	0.01	Cholic Acid	0.1
DL-Isoleucine	1.0	Silicon	0.046	Riboflavin	0.001
L-Leucine	1.5	Magnesium	0.048	Vitamin K	0.001
L-Lysine. HCl	1.4	Aluminum	0.008	Thiamine. HCl	0.001
DL-Methionine	0.6	Iron	0.014	Calcium Pantathonate	0.003
L-Proline	2.0	Copper	0.001	Biotin	0.00001
DL-Threonine	1.3	Zinc	0.001	Pyridoxine	0.001
DL-Tryptophan	0.5	Iodine	0.0008	Nicotininc Acid	0.003
DL-Valine	1.5	Cobalt	0.0005	Pteroylglutamic Acid	0.0001
Phenylalanine	2.2			Cellulose	5.00

*Sardine oil contains 400 vitamin D and 3000 vitamin A Units.
Glucose is added in amounts to make up balance of 100 grams of diet.

TABLE III

Essential Amino Acids Requirements of Chicks, Rats and Upper Limits of Humans (70 kg. Body weight.)

Amino Acids	Chicken (9,10) Per cent	Rat (11) Per cent	Upper limit 70 Kg. Man (12) Per cent
Arginine	1.2	N.E.	N.E.
Glutamic Acid	5.0	N.E.	N.E.
Glycine	1.8	N.E.	N.E.
Histidine	0.8	0.4	N.E.
Isoleucine	1.0	0.5	2.94
Leucine	1.5	0.8	4.64
Lysine	0.9	1.0	5.88
Methionine	0.9	0.6	3.50
Phenylalanine	1.6*	0.7	4.13
Threonine	1.3	0.5	2.94
Tryptophane	0.5	0.2	1.82
Valine	2.2	0.7	4.13

*In the presence of Tyrosine, Phenylalanine requirement is somewhat reduced.
N.E.=Not Essential.

acids in its diet than other species, for both growth and maintenance. In Table IV we present comparative data of some of the amino acids of chicken muscle, whole egg, whole milk, and beef.

TABLE IV

Amino Acids Composition of the Crude Protein of Chicken Muscle, Whole Egg, Whole Milk and Beef.

Amino Acids	Chicken Muscle (10) Per cent	Whole Egg (10) Per cent	Whole Milk (2) Per cent	Beef (2) Per cent
Arginine	7.0	7.0	3.5	6.4
Histidine	2.3	2.2	2.4	3.9
Lysine	8.4	6.0	8.1	8.9
Methionine	3.4	4.5	2.2	2.5
Cystine	1.2	2.4	0.5	1.4
Phenylalanine	4.6	5.8	4.6	4.2
Tyrosine	4.3	4.8	5.5	3.4
Tryptophane	1.3	1.5	1.4	1.4
Threonine	4.7	4.9	4.8	4.5
Leucine	*	9.2	11.8	5.9
Isoleucine	*	8.0	6.5	3.8
Valine	*	7.3	6.2	4.3

*Values have not been estimated.
Values are not available for other amino acids not listed.

Proteins are considered as primary constituents of structural and connective tissues of animals and are also very prevalent in the plant kingdom where they are particularly concentrated in seeds. They differ considerably from one another in their chemical structure, properties and in their amino-acids content. Amino acids are the simple units of proteins and impart to a protein its nutritive value in animals' and humans' dietaries. Unless a protein contains among its constituents all essential amino acids and can be properly digested by the animal system, and its constituents can be liberated in the digestive tract and absorbed, it cannot be utilized to form the many characteristic tissue proteins. One of the outstanding and astonishing phenomena of life is the unerring accuracy with which a specific tissue cell builds up a specific tissue protein out of the mixture of amino acids circulating in the blood (Rose).

The average diet of the chick contains about 20 per cent protein. In view of the well established large assortment of indispensable amino acids this species requires for growth and for maintenance, its diet is made up of plant and animal proteins. Whereas such nutrients are especially compounded to serve as good feed for chickens, many observers have recently found that the supplementation of feed with certain pure amino acids effects considerable improvement in growth, a reduction in feed consumption and a decrease in the rate of mortality. Poultrymen must become aware of these findings, which are indeed of considerable economic importance, if they desire to produce more and better chickens, induce hen to lay more eggs and realize greater profit.

The fact that the protein content of a good feed mixture contains all amino acids indispensable to the chick does not often guarantee optimum utilization and growth. The amino acid content of the diet must consist of a definite pattern and if any one of the indispensable amnio acids is quantitatively below the minimum requirement, then the biological principle, as we now understand it, is violated, because, the *deficient amino acid* in the diet becomes the *limiting factor* which governs the rate of growth of the animal. In other words, the animal system cannot synthesize more proteins unless all

the quantitative relationships of different amino acids that are essential to growth are found in definite proportions in the feed. As an analogy, let us consider the synthesis of water. We know that both oxygen and hydrogen are needed. These two gaseous elements are then the two indispensable elements. We also know that two molecules of hydrogen and one molecule of oxygen unite to produce one molecule of water. Let us therefore assume that we have two gram molecules of hydrogen and an unlimited amount of oxygen. Then irrespective of how much oxygen we may have we can produce only one molecule of water. The limiting factor in this instance is *hydrogen*. Let us also consider the synthesis of sulfuric acid. We need hydrogen, sulfur, and oxygen. If the availability of sulfur is limited and both oxygen and hydrogen are at hand in unlimited amounts, then we can synthesize sulfuric acid only to the extent of available sulfur. This *sulfur* in this instance is the limiting factor. In the protein molecule the unit of structure is an amino acid. The animal system has the ability to synthesize its various proteins from the amino acids in the diet. In effect it can function more efficiently, than we actually realize, as it can synthesize certain amino acids even though they may not be found in the diet. However, there are certain ones that the animal system cannot synthesize, probably due to peculiarities in their chemical structure. These amino acids are termed *indispensable* or *essential*. Those that are essential to the chick are listed in Table III and from this table we also note the quantitative requirement of this species and of those needed by man or by the white rat.

In feed mixtures, we undoubtedly have all indispensable amino acids required by the chick. The evidence is that the chick grows on such feed. The main question is: Do chickens obtain the maximum benefit from feed as far as its amino acid composition is concerned?

The quality of a feed manifests itself in the ability of the poultry to convert it into meat at the highest efficiency conversion factor. Let us take two feed mixtures A and B, and accordingly divide a flock into two groups, A receiving feed A, and B receiving feed B. If, on weighing the birds at the

end of 12 weeks, we discover that each bird in group A consumes, on an average, 4 pounds of feed per one pound of meat, and each bird of group B consumes only 3 pounds of feed B per one pound of meat, it becomes apparent that feed mixture B is the better of the two. Assuming the cost of the two feeds and other factors to be the same, it is obvious that mixture B is not only superior to A but is considerably more economical. Thus the conclusion is that mixture B permits greater production at a lower cost than mixture A and any poultryman who fails to use it cannot profitably operate his business.

Methionine

Almquist has shown that chicks require for optimum growth 0.9 per cent methionine (see Table III). It is also well established that poultry feed consists of a mixture of feedstuffs, preferably at 20 per cent protein level. It is therefore of paramount importance to know whether or not such mixtures contain the optimal amount of 0.9 per cent methionine. Should the diet of the chick contain less, then the feed would possess a low biological value, hence a low efficiency conversion factor. In order to correct this deficiency it becomes mandatory to supplement such mixtures with the necessary amount of methionine.

The data presented in Tables V and VI show the methionine content of crude proteins of certain feedstuffs of animal and plant origin. Values for methionine of the crude proteins in these two tables are given according to Grau and Almquist. At first glance most proteins of animal origin appear to contain ample methionine to satisfy chicks' requirement. However, these animal proteins are usually incorporated in the feed mixture to the extent of 5 or 10 per cent of the protein content of the diet. We must therefore consider their relative methionine content in relation to the percentages used in the diet. For a clearer interpretation, the methionine contents of 20 per cent, 10 per cent and 5 per cent protein levels are calculated and recorded in Tables V and VI. If the protein content of the diet were exclusively derived from animal proteins at 20 per cent level, the average methionine content would

TABLE V

The Methionine Content of Certain Crude Proteins of Feedstuffs of Animal Origin, Calculated at 20, 10 and 5 Per Cent Protein Levels.

Feedstuffs	Protein (Nx6.25) (14) Per cent	Protein Content (14) Per cent	Methionine content of feedstuffs 20 Per cent level Per cent	10 Per cent level Per cent	5 Per cent level Per cent
Blood fibrin, beef (Difco)	91.8	3.1	0.60	0.30	0.15
Blood fibrin, beef Swift	91.1	2.9	0.60	0.30	0.15
Blood serum, beef Swift	74.6	1.7	0.34	0.17	0.09
Casein	87.8	3.6	0.70	0.35	0.18
Fish meal, carp	58.6	2.7	0.54	0.27	0.14
Fish meal, sardine	69.6	3.1	0.60	0.30	0.15
Fish meal, shark	64.3	1.4	0.28	0.14	0.07
Fish meal, tuna	63.2	3.0	0.60	0.30	0.15
Fish press water, (menhaden, dried)	60.8	0.90	0.18	0.09	0.05
Fish press water, (sardine, dried)	66.5	1.90	0.40	0.20	0.10
Gelatin	95.1	0.40	0.08	0.04	0.02
Liver beef, dried	66.6	2.70	0.54	0.27	0.14
Meat scrap	67.3	2.0	0.40	0.20	0.10
Whey	11.0	2.1	.....	0.21	0.10
Averages:			0.42	0.21	0.11

only amount to 0.42 per cent. This therefore represents a methionine deficiency of 0.48 per cent. On the other hand, should half of the protein of the feed be derived from that of animal proteins, it would only contribute on an average of 0.21 per cent methionine. The remaining 0.69 per cent of methionine should then be derived from feeds of plant origin. The data found in Table VI show that the average methionine content of certain plant proteins at the 10 per cent protein level amounts to about 0.24 per cent. Russell *et al.* have shown that the average methionine content at 10 per cent protein level of 17 varieties of peas and beans is only 0.16 per cent. It is therefore unlikely that if half of the protein of the feed mixture is derived from that of plants it would contribute more than 0.24 per cent methionine. Thus the total methionine found in a feed mixture would only be 0.45 per cent at 20 per cent protein level. This clearly indicates a definite methionine deficiency which can only be met by methionine supplementation. In this connection one must recall that

although cystine is not indispensable to the chick, its presence in the diet is useful. However, the cystine content of most feedstuffs is indeed low, hence it can only partially and to a limited extent alleviate methionine deficiency.

TABLE VI

The Methionine Content of Certain Crude Proteins of Feedstuffs of Plant Origin, Calculated at 20, 10 and 5 Per Cent Protein Levels.

Feedstuffs	Protein (Nx6.25) (14) Per cent	Methionine Content of Protein (14) Per cent	20 Per cent level Per cent	10 Per cent level Per cent	5 Per cent level Per cent
Alfalfa meal	18.5	2.3	.....*	0.23	0.12
Barley	10.4	2.1	.....*	0.21	0.10
Corn gluten meal	45.6	2.3	0.46	0.23	0.12
Cottonseed	43.9	2.1	0.40	0.21	0.11
Fermentation residue	26.4	3.7	0.74	0.37	0.18
Linseed meal	35.1	2.3	0.46	0.23	0.12
Peanut meal	42.7	1.7	0.34	0.17	0.08
Sesame meal	47.8	3.4	0.68	0.34	0.17
Soy beans, raw	37.7	2.0	0.40	0.20	0.10
Soy bean meal	46.0	2.2	0.44	0.22	0.11
Sun flower seed meal	46.7	3.9	0.80	0.39	0.40
Wheat	10.8	2.0	.....*	0.20	0.10
Wheat protein, hydrolyzed commercial	48.6	4.4	0.88	0.44	0.22
Wheat bran	19.1	1.3	.....*	0.13	0.07
Zein commercial	91.6	1.6	0.37	0.16	0.08
Averages:			0.54	0.24	0.12

*Methionine values of Feedstuffs containing less than 20 per cent proteins were not calculated at 20 per cent protein levels.

Recent studies (Peterson, Russell, Clandinin) have shown that by supplementing feed mixtures with this sulfur-containing amino acid to the extent of 0.2 per cent of the feed, better growth of chicks are obtained, hence a higher efficiency conversion factor. Johnson has recently demonstrated that chicks kept for 12 weeks on an excellent feed mixture of natural sources, consumed at the rate of 3.4 pounds of feed per one pound of live meat, or an efficiency conversion factor of 29.5 per cent. By supplementing the same feed with 0.1 per cent DL-methionine, the average 12-week-old chick consumed only 2.8 pounds of feed per one pound of live meat or an efficiency conversion factor of 36 per cent. Noteworthy, also, was his

observation that there was a 50 per cent decrease in the rate of mortality of birds kept on feed supplemented with DL-methionine.

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SOME OBSERVATIONS ON THE EFFECT OF CYTOCHROME C IN RICKETTSIAL INFECTIONS*

JOE BENINSON AND LUDWIK ANIGSTEIN

Differences of opinion concerning the possible *in vivo* effect of cytochrome C make it seem worthwhile to report on the following observations made during the summer of 1947.

Proger *et al.* (1945a, 1945b, 1946, 1947) had reported the favorable clinical effect of cytochrome C therapy in anoxic conditions and the increased cytochrome C content of organs following parenteral injection. Keilin (1925) and others had shown that this portion of the cytochrome-cytochrome oxidase system increased the respiratory rates of certain mammalian tissues *in vitro*. Coupling these observations with the report of McLimans and Grant (1944) which referred to the finding that high oxygen tension therapy had been beneficial in experimental rickettsial infections (Scrub Typhus) it seemed reasonable to investigate the possible effects of cytochrome C in experimental typhus and Rocky Mountain spotted fever in guinea pigs. If cytochrome C were to induce a Pasteur effect intracellularly, perhaps the balance of the cellular enzyme systems could be shifted to the extent of making it unfavorable for rickettsial habitation.

The data presented below was obtained before the negative reports of other workers (Stadie and Marsh, 1947; Michel and Scheinberg, 1947; Benjamin, Steiner, and Milman, 1948; and Miller and Anderson, 1948), and the exchange of viewpoints, concerning the *in vivo* effectiveness of cytochrome C between Potter (1947) and Proger, *et al.* (1947) were published.

Materials and Methods

Animals. Guinea pigs of both sexes were obtained from Rockland Farms and fed dry pellets supplemented by carrots and lettuce.

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Cytochrome C. A granular beef heart preparation supplied by Endo Products, Inc. (N.Y.) and kept at 4° C. was dissolved in 50 cc. of 0.85% NaCl (1. mg./cc.) and filtered first by gravity through a coarse filter paper and then by vacuum through a sterile fritted glass filter (UF) before being placed in a sterile 50 cc. vaccine bottle. Forty-eight hour cultures on enriched blood agar plates and in enriched broth incubated at 37° C. remained sterile.

Preliminary determinations of the cytochrome C content on a Cenco-Sheard Spectrophotelometer by the extinction coefficient method described in Umbreit, Burris, and Stauffer (1945) using 13,000 as the molecular weight of cytochrome C, gave a value of 0.192 mg./mg. of granular preparation. Biological activity as ascertained in the Warburg apparatus using 1 cc. albino rat (250 gm.) liver brei, 1. cc. cytochrome C (1. mg./cc.) and a total of 1. cc. of buffer (pH 7.4) and 20.% KOH (CO_2 absorbent) gave a preliminary average oxygen uptake of 41.7 mm. of O_2/15′/mg. of granular preparation.

Cytochrome C administered in experiments I, II, and V was prepared as stated above using greater concentrations and when this sample was exhausted a Sharp & Dohme purified soluble beef heart cytochrome C was used with the same diluent for experiments III, IV, and VI.

Rickettsiae. The Cairo strain of louse-borne typhus was obtained through the courtesy of Dr. John P. Fox, Laboratories of the International Health Division, Rockefeller Foundation, New York, and the Rocky Mountain spotted fever strain was secured from Dr. R. R. Parker, Director, Rocky Mountain Laboratory, U. S. Public Health Service.

Mode of Administration. The dosages are stated in terms of the granular preparation (Endo) for which the preliminary analysis has been given with the exception of the aforementioned instances where the Sharp & Dohme preparation (unanalyzed) was used.

In these experiments all cytochrome C injections were given intraperitoneally using 0.85% NaCl as diluent and all solutions were checked for sterility as already mentioned.

The typhus and spotted fever inocula were prepared from sterility checked frozen infected brain (typhus) and spleen

(spotted fever), respectively, using 10% suspensions in skim milk and straining through double thicknesses of surgical gauze. One cc. of these suspensions was given intraperitoneally to each of the test animals as indicated.

Experiments and Results

These tests were designed for tentative answers to the possible effectiveness of cytochrome C in experimental rickettsial infections in guinea pigs. Three approaches were utilized: (a) infection on the same day the cytochrome C was begun, (b) infection on the third day after cytochrome C injection had begun, and (c) starting cytochrome C therapy on the initial day of fever.

Experiment I. Six guinea pigs averaging about 500 gm. each were infected with the spotted fever as described. One hour later four of them were given 4.0 cc. of cytochrome C (4. mg./cc.) intraperitoneally and this was repeated daily for a total of seven days (daily dosage 30 mg./Kg.). Both controls became febrile 48 hours after infections whereas the treated group remained afebrile for 3, 4, 4, and 5 days, respectively, representing an apparent lengthening of the incubation period; however, this was followed by typical spotted fever.

Experiment II. Four guinea pigs of another group of six animals averaging 450 gm. each, were infected with spotted fever on the third day following the initiation of 2.0 cc. cytochrome C (2. mg./cc.) injections which was continued for 9 days. The controls were febrile 48 hours after infection, whereas the treated were afebrile for 2, 3, 4, and 4 days, respectively, before coming down with typical spotted fever.

Experiment III. Six out of eight 400 gm. guinea pigs infected with spotted fever were given 2. cc. of cytochrome C (6.25 mg./cc.) on the first day of fever and again on the following day without any observable effect on the course of the disease.

Experiment IV. Paralleling Exp. III, Six out of eight 400 gm. guinea pigs infected with typhus were given 2. cc. cytochrome C (12.5 mg./cc.) for two days beginning on the first day of fever with no effect on the febrile course of the infection.

Experiment V. Six animals of a group of nine guinea pigs, weighing 400 gm. each were infected with typhus on the third day after the initiation of 2. cc. cytochrome C (3. mg./cc.) which was repeatedly given for a total of 11 days. The three controls were afebrile for 6, 6, and 7 days, respectively, whereas the treated animals were afebrile for 6, 6, 6, 7, 9, and 9 days individually, which is no change in the incubation period of this disease.

Experiment VI. Histologic sections taken from the liver of a 400 gm. guinea pig which had been given 3.25 mg. of cytochrome C daily for an 8-day period showed an essentially normal liver with considerable glycogen storage in the hepatic cells. Liver sections taken from a 500 gm. guinea pig which had received 16. mg. of cytochrome C daily for 7 days showed a diffuse fatty degeneration of the liver with no evidence of glycogen storage.

Discussion and Conclusions

Although the number of animals used in these experiments is not statistically great, they seem to indicate an overall trend.

It is felt that the slight prolongation of the incubation period observed in the spotted fever infected guinea pigs undergoing cytochrome C therapy can possibly be attributed to a non-specific physiologic action or a specific protein effect or both.

Gross pathology of the infected moribund animals which had been treated with cytochrome C, invariably showed pictures typical of the rickettsioses involved, however, the liver and lungs were pale, changing to normal color within five minutes after exposure to the air.

In one instance where a cytochrome C injection was accidentally made subcutaneously it was noted 24 hours later that the abdominal region was highly fibrosed around the injection site.

The cytochrome C treated infected animals did not seem to show any adverse effects clinically. In fact, they appeared to be more alert and active than the controls during the early part of the febrile period before displaying the classical late symptoms of spotted fever.

The negative findings of this report can possibly be explained by Stadie and Marsh's (1947) calculations (based on the turnover number of cytochrome C of Rosenthal and Drabkin) indicating that the amount of cytochrome C present in the cells of various albino rat tissues is physiologically compatible with its role in the oxidase-cytochrome system and the findings of Keilin (1925) on the common wax-moth which would indicate that the cytochrome C content of intact cells *in vivo* is sufficient even in extreme exertion. This coupled with Elliott and Greig's (1938) report that cytochrome C does not increase the O_2 uptake of intact tissues *in vitro*—although it does in homogenates, would indicate that injections of cytochrome C probably do not enter the cell *in vivo* in the manner hoped for.

This is also borne out by Benjamin, Steiner, and Milman's (1948) *in vivo* finding that 1.9 mg. intraperitoneal injections of cytochrome C given one hour before experimentally induced anoxia in guinea pigs less than 24 hours old is ineffective in combating anoxia and the experiments of Miller, Anderson, and Dorfman (1948), Stadie and Marsh (1947), and Michel and Scheinberg (1947) who found that pretreatment with cytochrome C did not prevent the decrease of readily hydrolyzable phosphate induced by anoxia. These reports would support the inability of cytochrome C to become functional in the oxidase-cytochrome system following injection.

Autopsies of treated animals failed to disclose any observable evidence that cytochrome C was being left behind in the abdominal cavity and it is suggested that this may be due to macrophagic activity which could also possibly explain Proger's (1947) observation that the increased organ content of cytochrome C was greater than that accountable for by the amount injected into the circulation. Activation of the reticulo-endothelial system could possibly fit in with the suggested nonspecific prolongation of the latent period observed in the spotted fever infected animals treated with cytochrome C.

In the light of the discussion and the findings, it would be of theoretical interest if the mechanism behind some of these observations could be elucidated; however, it is felt that no

intracellular Pasteur effect could be induced by cytochrome C in typhus and spotted fever. Greiff and Pinkerton (1948) have suggested that PABA, which is rickettsiostatic, may act through an increased cellular respiration. It is doubtful that any agent like cytochrome C, which is an integral part of the intracellular oxidative mechanism, will enter the cell following injection and alter the enzymic balances thus counteracting the intracellular disease agents.

In the discussion evidence was presented to favor the outlook that cytochrome C does not enter the cell, *per se*, to become functional in the oxidase-cytochrome system following injection.

Summary

1. The apparent lengthening of the incubation period of Rocky Mountain spotted fever in guinea pigs following intraperitoneal injections of cytochrome C of varying concentrations as indicated has been postulated as being a nonspecific physiologic effect or a protein effect or both.

2. Cytochrome C therapy begun on the day of fever in typhus and Rocky Mountain spotted fever in relatively large doses for two consecutive days did not alter the course of the fever.

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INFLUENCE OF ANTI-ORGAN SERA UPON METABOLIC PROCESSES

I. Reticulo-Endothelial Immune Serum (REIS) and the Oxygen Uptake of Rat Spleen

Wiktor W. Nowinski*

Pomerat and Anigstein (1944) applied strong concentrations of reticulo-endothelial immune serum (REIS) to tissue cultures *in vitro* and observed markedly reduced migration and clumping of cells of adult guinea pig spleen. They showed, furthermore, that this phenomenon is directly proportional to the concentrations of REIS and that it is species and organ specific. In another report (1945) the same authors not only confirmed the previous results, but showed that the same effect could be obtained, using homologous sera, on chick, rat, and guinea pig spleens, embryonic as well as adult. Pomerat (1945) demonstrated that strong concentrations of REIS also have an inhibitory action upon the migration of malignant cells (Walker Rat Sarcoma No. 319). From all these results the conclusion was drawn that there is a definite action of REIS upon tissues *in vitro*.

Anigstein and Pomerat (1945) also demonstrated the influence of REIS *in vivo* by producing bartonellosis and the accompanying anemia in rats. Frieden, Pomerat, and Anigstein (1945) and Pomerat, Frieden, and Yeager (1947) found the active principle located in the globulin fraction of this anti-organ serum. From this it follows that the inhibitory action of REIS, like that of the other anti-organ sera, is due with all probability to an immunological phenomenon. There is, however, strong evidence that the activity of antibodies is similar to that of enzymes and that there is a connection between

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enzymatic and immunological phenomena (Sevag, 1945). It was decided to investigate whether the inhibitory action of REIS, as manifested morphogenetically, is due to the inhibition of cellular oxidations. This point seemed particularly interesting because with the injection of bone marrow and spleen as antigens, all the cellular enzymes are injected as well and may act as antigens in producing anti-enzymes.[1]

Methods

For the measurement of oxygen uptake, Warburg technique was used. The slices and homogenates of rat spleens were placed in standard Kremer flasks and in order to increase the absorption surface for CO_2, small rolls of filter paper were placed into the center well for alkali (Dixon, 1933).

In the first series of experiments a calcium-free Tyrode was used, which was substituted later by Ringer for mammals, mixed 1:1 with phosphate buffer. The final pH of the solution was 7.2. Glucose was not added as substrate because it is contained in sufficient quantities in serum already.

The experiments usually were run for 60-90 minutes, the readings being taken every 15 minutes. After the experiment, the slices were washed in distilled water, dried in an oven at 105° C., and the dry weight estimated. In the case of suspensions, 1 ml. was taken for drying and the Q_{O_2} was calculated accordingly. The temperature of the Warburg bath was 38° C. throughout the experiments.

The anti-rat REIS was prepared as described by Anigstein, Whitney, Pomerat, and Orr (1947) and the complement fixation titer was determined.[2]

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[1] When the experimental part of this work was completed, a paper by Cohen, Runjavac, and Strauss (1947) was published, in which the authors demonstrated that REIS has no effect upon respiration of bone marrow suspensions of guinea pigs. As the present paper deals with rat spleen and uses a somewhat different technique, Cohen *et al.* and our results complement each other.

[2] We wish to express our thanks to Miss Mary Faith Orr and Miss Doorthy Ezell for the preparation of the Reticulo-Endothelial Immune Sera. Thanks are due also to Miss Dorothy Whitney for the titration of sera used in our experiments.

As controls, normal rabbit sera were used in concentrations corresponding to that of REIS.

Results

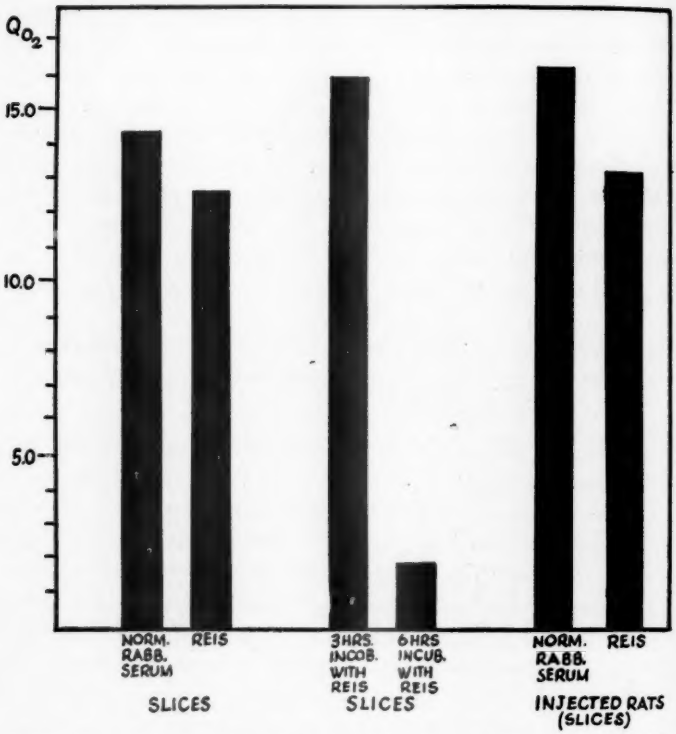
In the first series of experiments, the influence of REIS was investigated by adding it directly to the reaction flask. In order to obtain a strong effect, a final concentration of 1:4 of serum in Tyrode or Ringer was used. The results of these experiments are summarized in Table I and in Figure 1 (A). In

TABLE I

Oxygen Uptake of Rat Spleen Slices in Normal Rabbit Serum and in REIS

Normal Rabbit Serum Q_{O_2}	REIS Q_{O_2}
—13.94	—10.96
— 4.72	— 8.80
—10.52	— 6.87
—19.55	—14.28
—12.00	— 7.27
—12.90	—16.78
—11.09	— 9.21
—15.30	—12.44
—20.64	—13.90
—21.05	—15.86
—16.50	—15.21
—18.61	—12.82
—20.00	—10.78
—19.70	—13.49
—12.46	—11.62
— 9.32	—11.49
—14.48	—16.66
— 9.80	—11.82
—17.26	—21.30
—13.29	
—11.61	
—10.95	
Average —14.33	Average —12.64

spite of the great divergency in the single quotients, the average Q_{O_2} does not show a marked difference: —14.33 for normal rabbit serum against —12.64 for REIS. In order to check these results, attempts were made to establish whether or not by changing the concentrations of REIS a correlation between



inhibition and concentration could be found. Figures obtained in these experiments are listed in Table II which shows that

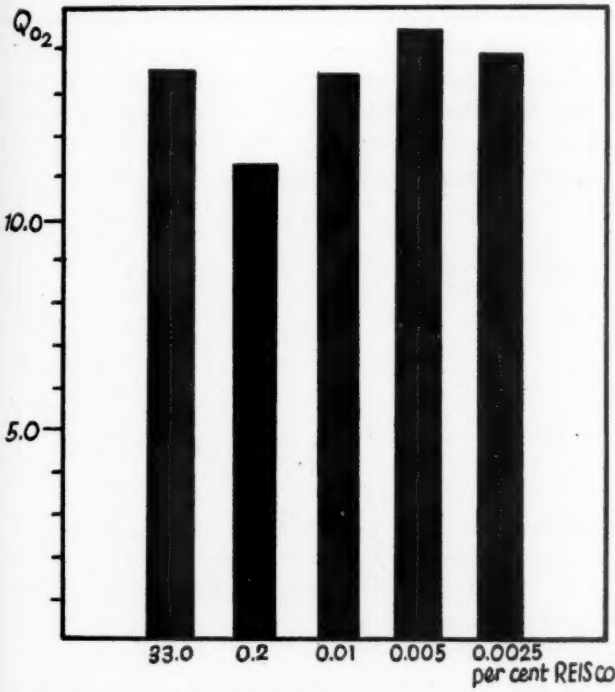
TABLE II

Influence of Decreasing Doses of REIS Upon Oxygen Uptake of Rat Spleen Slices.

Final concentr. of REIS	33%	0.2%	0.01%	0.005%	0.00025%
	—13.79	—11.06	—11.18	—12.66	—11.19
	—15.09	—10.83	—10.52	—10.13	— 9.33
	—11.90	—10.69	—10.08	—11.16	—14.21
		—11.97	—15.70	—17.07	—14.77
			—15.02	—19.31	—20.01
			—18.22	—17.10	
Average Q_{O_2}	—13.59	—11.13	—13.45	—14.56	—13.90

no great difference between the average quotients exists: —13.59 for a 33% final concentration of REIS, 11.13 for 0.2%, —13.45 for 0.01%, —14.56 for 0.005%, and —13.90 for 0.0025%. Results shown graphically in Figure 2 demonstrate these negative findings particularly clearly.

At this stage of the work, the supposition was made that possibly the effect of REIS manifests itself after a certain period of time, i.e., after incubating with slices. In experiments which lasted for three hours, no effect could be observed either, as seen from Figure 3: it shows the rate of oxygen



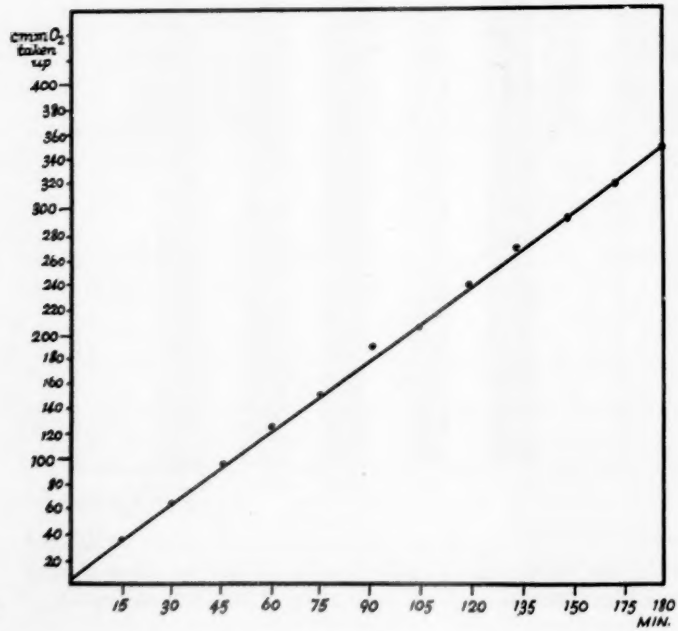
uptake by tissues in a single flask, expressed in cubic milliliters of O_2; the curve rises steadily and there are no signs of any inhibition whatsoever. As seen from Table III, the average

TABLE III

Influence of REIS Upon Oxygen Uptake of Spleen Slices as a Function of Incubation Time

After 3 hours of incubation		Q_{O_2}
		—18.70
		—15.61
		—16.11
		—13.33
		—15.72
	Average	—15.89
After 6 hours of incubation		
		— 2.02
		— 1.90

Q_{O_2} was —15.89, which corresponds to the figure obtained in previous experiments with normal rabbit serum. In cases where the slices were incubated with REIS for six hours and then the oxygen uptake measured, the average Q_{O_2} is rather



low (—1.96), this low value, however, cannot be attributed to the inhibitory action of REIS, but rather to autolysis of the tissue slices (toluene in sufficient quantities was added in order to prevent bacterial development). This assumption was confirmed by their general microscopic aspect which suggested some far-reaching changes. The values for the last two series of experiments are listed in Figure 1 (B) from which a comparison with the normal rabbit serum and REIS may be made.

Because of the negative results obtained with sera added *in vitro*, it was decided to investigate the oxygen uptake of spleens of rats previously injected with high doses of REIS. The animals were killed 3 days after the injection, because it was shown by Anigstein and Pomerat (1945), and Pomerat, Frieden, and Yeager (1947) that at this time the anemia produced by high doses reaches its maximum, *i.e.*, that there is a maximum injurious action of REIS in Bartonella infected rats, *in vivo*. The results of this series of experiments are shown in Table IV and Figure 1 (C). The average Q_{O_2} is —13.25 for

TABLE IV

Oxygen Uptake of Spleen Slices of Rats Previously Injected with REIS, and with Normal Rabbit Serum as control.

Normal Rabbit Serum Q_{O_2}	REIS Q_{O_2}
—14.72	—16.34
—14.60	—10.59
—19.62	—12.82
Average —16.31	Average —13.25

rats injected with REIS, and —16.31 for rats treated with normal rabbit serum. This amounts to a difference of about 18 per cent, which is not considered significant.

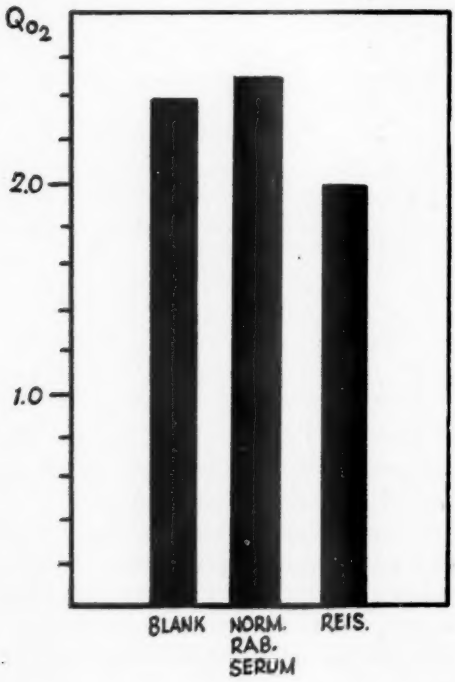
Since all the series of experiments reported yielded no positive inhibitory effect upon oxygen uptake of the spleen, the question arose as to whether under the conditions of the present experiment, the antibodies penetrate inside the cell at all. In order to check this possibility, experiments were set up with

spleen suspensions. Table V and Figure 4 show the data obtained. The average quotient for the suspension alone was —2.39, against —1.99 for suspension with added REIS.

TABLE V

Oxygen Uptake of Spleen Homogenates without Serum, with Normal Rabbit Serum and with REIS.

	No Serum Added Q_{O_2}	Normal Rabbit Serum Q_{O_2}	REIS Q_{O_2}
	—3.12	—2.79	—3.11
	—2.46	—1.76	—2.62
	—2.13	—2.82	—1.21
	—1.85	—1.89	—1.92
	.....	—2.95	—1.10
Average	—2.39	—2.44	—1.99



Discussion

From these experiments it follows that, as far as cellular oxidations are concerned, no inhibitory effect of REIS upon spleen slices and suspensions could be demonstrated. Although in some cases there seemed to be a certain decrease in oxygen consumption, as in the case of slices in normal rabbit serum and in REIS, or in rats previously treated with REIS, the differences are much too small to account for such marked morphogenetic changes as those observed by Pomerat and Anigstein (1945) and Pomerat (1945). That the inhibitory activity is not manifested even after a prolonged incubation, was shown in the "time experiments" where the tissues were incubated for 2 and 6 hours, respectively.

It was also found that there is no relation between the oxygen uptake and the titer of the REIS (as demonstrated in *in vitro* experiments by Pomerat). In Table VI the corresponding data are put together. From this table follows that the Q_{O_2} for slices treated with REIS of a compliment fixation titer 1:1000 is equal to that of slices treated with a REIS of a titer 1:320 (—11.84 and —11.43) and do not differ very much from the Q_{O_2} of slices with REIS of a titer 1:160 (—13.28). These results once more confirm the fact that REIS does not interfere with the cellular oxidations.

Table VI

Relations Between Complement Fixation Titer of REIS and the Oxygen Uptake (Q_{O_2}).

Titer	1 : 1000	1 : 320	1 : 160
	— 8.80	— 6.38	—12.62
	—14.28	— 7.27	—15.86
	—12.44	—13.90	—15.21
		—10.96	—10.78
		—17.21	—13.49
		—13.29	—11.63
		—11.01	—11.49
		—10.95	—16.66
			—11.82
Average	—11.84	—11.43	—13.28

That permeability does not play a role was shown by the oxygen uptake of spleen suspensions with normal rabbit serum and with REIS. The general decrease in the Q_{O_2} was rather due to the destruction of the cell as a system, as shown by the lowering of the quotient for the suspension alone (—2.39) and that with added normal rabbit serum (—2.44), whereas the corresponding figure for slices is —14.33 with added normal rabbit serum. The lack of inhibitory activity of REIS is particularly well manifested in experiments with decreasing concentrations of REIS (Table II and Figure 2): if even the slightest inhibition were provoked, there should be a definite parallel between the inhibition and concentration. These experiments fully confirm the conclusions drawn by Cohen, Runjavac, and Strauss from their investigations on the influence of REIS upon bone marrow. It is obvious therefore that the morphogenetic effects obtained with REIS *in vitro* are not due to changes in cellular oxidations, but, possibly, to changes in another enzyme system or systems.

Summary

The influence of strong doses of REIS upon oxygen uptake of rat spleen slices and suspensions was studied. No inhibitory effect of the REIS could be observed, as compared with the figures for slices and suspensions when normal rabbit serum was added. The morphogenetic phenomena observed in tissue cultures are not, therefore, due to an inhibition of cellular oxidations.

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YOLK SAC CULTIVATED TUMOR TISSUE AND EXPERIMENTS IN TUMOR CHEMOTHERAPY

I. Riboflavin

Alfred Taylor and Nell Carmichael*

Research directly concerned with the problem of finding substances which are more toxic to tumor tissue than to rapidly growing nontumor tissue has been relatively limited. A search through the literature discloses that a large volume of data has been accumulated on various conditions and compounds which initiate the cancer process or increase the growth of neoplastic tissue. But very little has been accomplished in the search for materials which are selectively inhibitory to tumor growth or cause the destruction of tumor tissue. Where such reports have been made, considerable controversy has arisen and the evidence has assumed a doubtful character.

Most of the work done in the field of cancer control has utilized induced or transplant tumors grown in mice or rats. Under these conditions it is often difficult to dissociate effects on the tumor directly related to procedures and compounds used from indirect effects mediated through changes in the host animal. Even where the weight of the experimental mouse or rat is unaffected, changes in tumor growth rate or destruction of tumor tissue can not always be considered as a specific reaction to the chemicals or substances under consideration.

It is well-known that animals such as the rat or mouse are able to make rapid adjustments to the introduction of nontoxic substances. In addition to excretion through the kidneys, the chemistry of the injected compound may be changed. Often it is doubtful whether any appreciable concentration of a particular chemical which has been injected into the tumor bearing animal ever contacts the tumor tissue.

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Recently in this laboratory, cancer chemotherapeutic studies have been undertaken in which egg cultivated tumor tissue was utilized. This approach appears to have some advantages over the use of mice and rats at least for preliminary testing of possible anticancer substances. While much of the work to date has been exploratory, enough has been accomplished to demonstrate the utility of egg grown tumors in these studies.

The present paper is concerned with a description of the procedure involved in this research together with some of the results obtained.

Yolk Sac Cultivation of Tumor Tissue

The yolk sac method of tumor cultivation was initiated in this laboratory more than 6 years ago (Taylor, Thacker, Pennington, 1942; Taylor, Hungate, Taylor, 1943; Taylor, Carmichael, Norris, 1948), and has been utilized continuously since that time.

The number of eggs inoculated in this manner with cancer tissue numbers several hundred thousand and the technique has been very much improved with experience in its application. Individual strains of tumor tissue have been carried for more than 6 years by egg to egg transplant.

The yolk sac technique is much simpler than other methods of cultivating tumor tissue in eggs. It consists merely of the injection of a tumor suspension through the air space and into the yolk sac of embryonated eggs which have been incubated about 4 days. The tumor tissue introduced into the egg in this manner implants on the inner wall of the yolk sac and becomes vascularized with chick blood vessels of the area vasculosa (Hungate, Taylor, Thompson, 1944). The chick embryo and the yolk sac tumor grow together in the egg sharing a common blood supply but otherwise not interfering with each other.

The tumor tissue produced by the yolk sac method grows more rapidly in many instances than transplants of the same cancer tissue in the host animal. Further, when carefully done

the variation in size of these egg grown tumors is less than that associated with ordinary transplants. Mouse mammary cancer from two different strains, dba and C_3H mice, when cultivated in eggs regularly attains an average of 0.9–1.0 gram in size by the 15th day of incubation or 11 days after egg inoculation. These same tumors will average nearly 3 grams when carried to the 17th day of incubation or 13 days after egg inoculation.

Eggs inoculated by the yolk sac method develop tumors in every instance.

Finally the method of growing tumors by the yolk sac technique is relatively simple and rapid in execution. Around 60 dozen eggs are now being inoculated with tumor tissue each week. The whole process of inoculating, servicing, and harvesting these eggs requires about 2 hours a day, 6 days a week for 2 people. Yet out of this investment of time and materials there is available for experimental purposes each week some 40 dozen tumor bearing eggs. It is quite evident that this method furnishes tumor material much more cheaply both from the standpoint of expense and time than could be obtained by mouse transplants.

The Use of Egg Grown Tumors in Chemotherapy Studies

A technique has been developed by means of which chemicals can be deposited in contact with the living tissue of the chorioallantoic membrane of the embryonated egg analogous to a subdermal injection in a free living animal. It has been found that injections can be made so that the material used is placed between the shell membrane and the chick tissue. The process is very simple and quickly carried out and when inocuous substances are used has no ill effect on the embryo. Saline injection made in this manner does not increase the mortality over that of uninjected eggs.

The technique is being used in association with tumor bearing eggs to test out possible anticancer properties of various specific chemical compounds and other substances of unknown

composition. Numerous experiments involving several thousand eggs have been completed and many others are contemplated or are in progress. The procedure used at present is as follows:

Tumor bearing eggs incubated a total of 8 days at 99° F. or 4 days after tumor inoculation are used in these experiments. At this stage the tumor is well implanted along the sinus terminalis of the area vasculosa (Figure 1). At the same time the quantity of tumor tissue in each egg is relatively small.

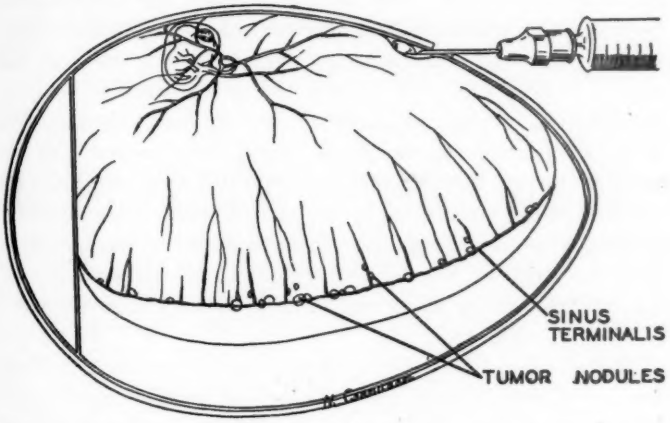

FIG. 1. Eight-day-old tumor-bearing egg showing needle in position for injection of materials between shell and chorio-allantoic membranes.

Several hours before treatment the eggs are placed in a horizontal position so that the embryo, area vasculosa and chorio-allantoic membrane will come to rest at the upper side of the egg. Each egg is candled and a small circle about 1/4 inch in diameter is made over the area vasculosa below the location of the embryo. The encircled places are cleaned and partially sterilized by swabbing with 70% alcohol. The eggs are now ready for the shell perforation. This can be done quickly and easily by gently tapping the indicated spot with the sharp point of a small pair of scissors. Only the outer shell is dented. Care must be taken not to break through the shell membrane.

The material for injection is prepared so that 0.1 to 0.2 cc. is inoculated into each egg. With a 1 cc. syringe and a 23 gauge needle, (a larger needle up to 20 gauge can be used but is less desirable) the quantity of sterile material to be injected is drawn up into the syringe. An egg is held in the left hand with the perforation upward and the needle inserted at a slant as shown in Figure 1. The needle is thrust under the shell just beyond the bevel of the point. The contents of the syringe are slowly expelled. The shell membrane fits around the needle so that there is no regurgitation of the injected solution. Before proceeding to the next egg the hypodermic needle of the syringe is dipped in 70% alcohol and passed through a flame. This is to insure against possible infection from minute pieces of egg shell which may break off around the edges of the perforation and stick to the needle as it is withdrawn from the egg.

The openings in the eggs are sealed with melted paraffin applied wtih a glass rod. The eggs are placed in the incubator with the injected side up and left undisturbed for about 24 hours after which they receive the usual treatment given incubating eggs.

The material injected into the egg in the manner described, forms a deposit between the shell and the corio-allantoic membranes (Figure 1). It is taken up by the membrane blood vessels and circulated through embryo and tumor tissue.

In the present study it is very important to introduce the test chemical into the egg in such manner that the chemical itself is the only new variable. The method given accomplishes the objective. Repeated tests with nontumor as well as tumor bearing eggs has demonstrated that the survival, growth and development of the embryos of the eggs injected with 0.85% saline is exactly the same as that of comparable untreated controls.

A method has been described by Burnet (1933) for the inoculation of the chorio-allantoic membrane with virus preparations which make use of an artificial air space. The regular air space is perforated and an opening is made over the chorio-

allantoic area of the egg. The second shell perforation results in the collapse of the original air space with the formation of another over the chorio-allantoic membrane. Material is injected into the artifical air space. Both openings in the egg shell are sealed over.

For the purposes of the present research Burnet's technique has been found to have certain disadvantages as compared with the simpler method described above. It subjects the egg to a type of manipulation which may interfere with the subsequent growth and development of the chick embryo.

The Use of B Vitamins in Tumor Chemotherapy

Several years ago in this laboratory an investigation was made of the B vitamin content of tumor and nontumor tissue (Taylor, Pollack, Williams, 1942; Pollack, Taylor, Williams, 1942). It was found that cancers of various types and from various animals including man tend to have a common pattern with respect to the concentration of the various B vitamins. Malignant tissue from the mouse, rat and man tended to be as uniform in this respect as cardiac muscle tissue from the same animals. The investigation disclosed that in general the level of these vitamins was lower in neoplasms than in nontumorous tissue. One exception to this was folic acid which proved to be relatively high and it was because of this report that Lewisohn and his associates (1947) used this vitamin in tumor chemotherapy studies.

It is well known that a compound essential to the physiology of a tissue may have toxic or adverse effects when present at abnormally high levels. Since the concentration of the various B vitamins differs as between tumorous and nontumorous tissue it was considered that there might also be differences in the reaction to high concentrations of these compounds. Accordingly studies of the possible use of the B vitamins in cancer chemotherapy have been undertaken using egg grown tumors.

In the present paper a report is given of the results obtained with riboflavin. The data obtained was negative but it will serve to illustrate the use of egg grown tumors in cancer chemotherapy studies.

Experiments with Riboflavin

Using the technique already described various quantities of riboflavin were injected into eggs bearing mouse mammary tumors in order to test the response of tumor and embryo tissue to high levels of this vitamin. Altogether 11 experiments involving 231 tumor bearing eggs were completed using levels of riboflavin which ranged from 0.01 to 5 milligrams per egg. In each experiment the eggs were divided into two equal groups, the one receiving the vitamin, the other, the control, receiving the suspending solution.

Riboflavin is relatively insoluble in water. In this work the vitamin was injected in form of a suspension in 0.85% saline solution and adjusted so that each egg was injected with 0.1 cc. of the suspension.

The results are summarized in Table I. It will be noted that there was no appreciable effect either on chick embryo or tumor size. Inspection of the figures on survival of the injected eggs reveals that even in the group receiving 5 mgs. of riboflavin per egg there was no indication of toxicity.

Summary and Conclusion

Cancer therapeutic studies have been undertaken utilizing tumors grown in eggs by the yolk sac method.

The compound to be tested is injected so as to form a deposit between the shell and the chorio-allantoic membrane of eggs bearing implanted tumor tissue. The procedure is analogous to a subdermal injection in a free living animal. Eggs subjected to this treatment survive as well as untreated controls.

Tumors produced by the yolk sac method develop and grow without interfering with the chick embryo. The effect of the test compound can be evaluated with respect to tumor and embryo tissue sharing a common blood supply.

Riboflavin in varying concentrations was used as the test chemical in a total of 11 experiments involving 231 tumor bearing eggs. There was no appreciable effect on embryo or tumor size. Riboflavin in 5 mg. doses was not toxic to 8 day chick embryos.

TABLE I

Effect of the injection of various quantities of riboflavin into 8 day old tumor bearing eggs on the size of tumor and embryo harvested at the 15th day of incubation.

	No. Eggs injected		Survival at harvesting (percent)		Tumor size (gms.)			Chick size (gms.)		
	Ribo-flavin	Saline Control	Ribo-flavin	Saline Control	Ribo-flavin	Saline Control	Riboflavin Control 100	Ribo-flavin	Saline Control	Riboflavin Control 100
			Experimental: .01–.1 mg. riboflavin in .85% saline. Control: .85% saline.							
	9	9	67	56	1.03	.74	139	7.8	9.0	87
	13	14	77	93	.80	.99	81	8.7	8.3	105
	10	11	80	82	1.15	1.07	107	7.1	7.6	93
	11	10	64	70	.87	.93	94	9.0	8.8	102
Total	43	44	Av. 72	75	.96	.93	105	8.2	8.4	97
			Experimental: .1–1.0 mg. riboflavin in .85% saline. Control: .85% saline.							
	5	5	60	80	.80	1.10	73	8.3	9.5	87
	9	10	44	50	.65	1.20	54	9.6	8.1	119
	10	11	70	82	1.06	1.07	100	7.9	7.6	104
	11	10	91	70	.87	.93	94	8.2	8.8	93
Total	35	36	Av. 66	71	.85	1.08	80	8.5	8.5	101
			Experimental: 1.0–5.0 mg. riboflavin in .85% saline. Control: .85% saline.							
	10	10	80	80	.97	.73	139	8.8	9.3	95
	12	12	92	91	.82	.86	95	8.9	7.8	114
	15	15	80	73	.57	.90	63	9.1	9.2	99
Total	37	36	Av. 84	81	.79	.83	99	8.9	8.8	103

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CALLING ATTENTION TO

Items of Possible Interest to Our Friends
September, 1948

1. Physioligical: M. I. Grossman and others offer proof of hormonal mechanism for gastric secretion following distention (*Am. J. Physiol.*, 153/1/48). K. J. O'Brink studies kinetics of parietal secretion of stomach (*Acta Physiol. Scand.*, 15, Suppl. 51, Uppsala, '48, 106 pp.). S. Wolf and H. G. Wolff estimate protective action of gastric mucin vs. corrosive chemicals (*Gastroenterol*, 10/251/48). J. R. Brobeck indicates food intake as part of mechanism for regulating body temperature (*Yale J. Biol. Med.*, 20/545/48). H. G. Bayze and C. Engel note activity of digestive enzymes on folic acid conjugates (*Biochim. Biophys. Acta.*, 2/217/48). D. A. Hall suggests enzymic metabolism of histidine is involved in folic acid enzyme system (*Nature*, 162/178/July/31/48, confirming C. A. Elvehjem and others, *J. Biol. Chem.*, 174/319/48). D. Barker describes innervation of muscle spindle (*Quart. J. Microsc. Sci.*, 89/143/48). E. B. Verney discusses function of pituitary pars nervosa (*Brit. Med. J.*, 2/119/July 17/48). T. I. Chen and C. Tsai describe mechanism of hemostasis in peripheral vessels (*J. Physiol.*, 107/280/48). C. D. deLangen discusses significance of liver in porphyrin metabolism (*Bull. Schweiz Akad. Med. Wiss.*, 6/385/48). C. A. Mills confirms need for more thiamin with age (*Am. J. Physiol*, 153/31/48). C. G. Lambie and B. D. Wyke discuss pathological physiology of pyramidal syndromes (*Med. J. Austral.*, 2/85/July 24/48). Our E. Ogden and E. Tripp show diuresis increases urea production (*Am. J. Physiol.*, 153/190/48).

2. Pharmacological: Folic acid free anti-pernicious anemia agent, vitamin B_{12}, shown by E. L. Rickes and hot Merck group to be a cobalt co-ordination complex (*Science*, 108?134-Aug. 6?48). C. P. Huttrer reviews chemistry of antihistaminic agents (*Enzymol.*, 12/277–332/48). B. Russell and others report latent tetany from BAL (dithiopropanol) (*Lancet*, 2/169/July 31/48). B. Folkow and others suggest two types of histamine sensitive receptors only one of which may be blocked by benadryl and related compounds (*Acta Physiol. Scand.*, 15/264/48). T. Savolainen studies growth inhibition of anaerobic bacteria by organic compounds (*Ann. Med. Exp. Biol. Fenn.*, 26, Suppl. 2, Helsinki, '48, 177 pp.). F. N. Fastier and F. H. Smirk note cardiac depressing action of amarin-triphenyldihydro-iminazole (*J. Physiol.*, 107/318/48). H. O. Bang and J. Georg show bulk of absorbed iodophthalein excreted in feces (*Acta. Pharmacol.*, 4/87/48). K. E. Penrod and A. H. Hegnauer show hemodilution and resp. depression from pentothal anesthesia may be prevented by epinephrin (*Am. J. Physiol.*, 153/81/48). K. I. Melville surveys anti-fibrillation effect of papaverine (*Rev. Canad. Biol.*, 7/236/48). L. H. T. Bernstein studies epithelial skin cells in relation to disinfection (*J. Invest.*

Dermatol., 11/49/48). R. Pratt and others note vitamin K_5 as inhibitor of growth of fungi and yeasts (*Proc. Nat. Acad. Sci.*, 34/323/48). J. Dufrenoy and others describe growth inhibiting effects of salicylacetone on dermatophytes (*Growth*, 12/157/48). H. Eagle and R. Fleischman report bacitracin synergizes with penicillin in action on syphilis (*Proc. Soc. Exp. Biol. Med.*, 68/415/48). G. C. Bond and others find bacitracin orally clears strept. and anaerobes from gut, but not coliforms, is absorbed and excreted in urine (*ibid.*, p. 395). J. L. Morrison and others show myanesin distributed according to water content with rapid metabolism of 1.5 mgm/kg/min (*ibid.*, p. 343). E. M. MacDonald and A. L. Tatum find detergents promising as trichomonacidal agents (*J. Immunol.*, 59/301/48). D. A. Karnofsky reports on chemotherapy of neoplastic disease (*N. Eng. J. Med.*, 239/226, 260/Aug. 5, 12/48). W. E. Cohn discusses toxicity of ingested radiactive products (*Nucleonics*, 3/21/48). G. N. El-Din notes toxic psychoses after atabrine (*J. Roy. Egypt. Med. Asso.*, 31/489/48). Our E. J. Poth discusses gut healing as influenced by intestinal antiseptics (*South. Med. J.*, 41/672/48).

3. Otherology: Noteworthy is *General Cytology* of E. D. P. DeRobertis, our W. W. Nowinski and F. A. Saez; well organized, illustrated, clearly written, simulating references, get it (Saunders, Phila., '48, 356 pp., $7). S. Giedion considers how *Mechanization Takes Command* (Oxford Press, N.Y., '48, 800 pp., $12.50). April or May issue *Indian Med. Gaz.* will be Tragedy Number in memory of Ghandi, with important social medicine contributions. J. F. Brock suggests new concepts in disease etiology with reference to disease prevention (*South Afr. Med. J.*, 22/440/48). F. A. E. Crew discusses social medicine as human ecology (*Acta. Med. Leg. Soc.*, 1/85/48). W. V. Mayneord describes organization of teaching and research in medical physics (*Acta. Radiol.*, 29/435/48). J. H. Lawrence and J. G. Hamilton fittingly edit Vol. 1, *Advance in Biological and Medical Physics* (Academic Press, N.Y., '48, 495 pp., $8). H. P. Klug notes new attempts to see molecules (*Am. Sci.*, 36/377/48). F. B. Seibert and others report serum polysaccharide of possible diagnostic aid in tuberculosis and cancer (*Arch. Biochem.*, 18/279/48). J. H. Young describes recognition and treatment of intervertebral disc disease (*N. Zealand Med. J.*, 47/106/48). D. Bodian discusses relations of polio virus to nerve cell and paralysis (*Bull. Johns Hopkins Hosp.*, 83/1/48). A. E. Barclay describes micro-arteriography (*Am. J. Roentg. and Rad. Therap.*, 60/1/48). S. Kallner discusses thrombosis as complication of internal disease (*Arch. Int. Med.*, 81/126/48).

October, 1948

1. Snappy Autumn Books: J. R. Newman sniffily reviews (*New Republic*, Sept. 20) I. B. Cohen's *Science, Servant of Man* (Little Brown, Boston, '48, 376 pp., $4), which contains many useful case histories. F. A. Willius and T. J. Dry compile important *History of the Heart and Circulation*, with unusual organization and illustration (Saunders, Phila., '48,

456 pp., $8). G. W. Corner edits Autobiography of *Benjamin Rush* (Princeton Press, '48, 512 pp., $7.50). P. H. Oehser tells story of Smithsonian Institution in *Sons of Science* (Schuman, N.Y., '48, 320 pp., $4). D. Stimson offers a history of the Royal Society in *Scientists and Amateurs* (Schuman, N.Y., '48, 220 pp., $4). H. V. Hentig spreads the blame in *The Criminal and His Victim* (Yale Press, '48, New Haven, 469 pp., $6). A. Smith and A. D. Herrick edit practical *Drug Research and Development* (Revere, N.Y., '48, 650 pp., $10). V. R. Potter edits important *Methods in Medical Research* (Year-Book, Chicago, '48, 372 pp., $8). C. Voegtlin edits timely *Pharmacology and Toxicology of Uranium Compounds* (McGraw-Hill, N.Y., '48, 550 pp., $10). W. Bloom edits equally timely *Histopathology of Irradiation from External and Internal Sources* (McGraw-Hill, N.Y., '48, 875 pp., $15). G. Hevesy offers *Radiactive Indicators: Their Application in Biochemistry, Physiology and Pathology* (Interscience, N.Y., '48, 572 pp., $9). W. H. Seegers and E. A. Sharp describe *Hemostatic Agents: Thrombin, Fibrinogen and Absorbable Cellulose* (C. C. Thomas, Springfield, Ill., '48, 143 pp., $4.50). Note symposium on *Rickettsial Disease of Man* (A.A.A.S., Washington, '48, 220 pp., $6.25). P. H. Van Thiel covers *The Leptospiroses* (Univ. Leyden Press, M. Nijhoff, The Hague, '48, 241 pp., 16.5G). H. D. Anthony snaps in *Science and Its Background* (Macmillan, London, '48, 313 pp., 10s6d). Sir Arthur Keith has *New Theory of Human Evolution,* claiming isolated inbred groups tend to diverge (Watts, London, '48, 461 pp., 21s). J. Maisin summarizes *Cancer: Heredite, Hormones. Substances Cancerigenes* (Casterman, Paris, '48, 248 pp., 84fr.). M. D. Leigh and M. K. Belton's *Pediatric Anesthesia* is useful (Macmillan, N.Y., '48, 255 pp., $5.50). Imposing is J. A. Myer's and C. A. McKinlay's *The Chest and the Heart* (C. C. Thomas, Springfield, Ill., '48, 2 vols., 1856 pp., $25). Useful is C. C. Sturgis's *Hematology* (C. C. Thomas, Springfield, Ill., '48, 935 pp., $14).

2. PHYSIOLOGY: H. C. Bazett and others fittingly open new periodical with report on heat exchange in blood flow (*J. Appl. Physiol.*, 1/3/48). D. H. K. Lee and R. K. MacPherson study tropical fatigue (*ibid.*, p. 60). G. H. Bishop and others report patterns of tremor in normal and pathological states (*J. Appl. Physiol.*, 1/123/48). W. Eger discusses liver histology in relation to variations in glycogen, protein, fat and nitrogen (*Virchow's Arch. Path. Anat.*, 315/135–172/48). M. E. Hansen, M. I. Grossman and A. C. Ivy discuss histamine gastric secretion emphasizing sensitivity of parietal cells (*Am. J. Physiol.*, 153/242/48). M. Fabinyi and J. Szebehelyi find histamine increase in oxygen lack (*Arch. Internat. Pharmaco Therap.*, 76/397/48). D. R. Wood finds caffeine and related compounds potentiate histamine on gastric secretion (*Brit. Med. J.*, 2/283/Aug. 7/48). E. W. Sutherland and C. de Duve describe glycogenolytic factor from alpha cells of pancreas islet tissue (*J. Biol. Chem.*, 175/663/48). W. F. Hamilton and others discuss factors regulating stroke volume of heart, and find Fick and dye methods for cardiac output agree (*Am. J. Physiol.*, 153/287, 298, 309/48). R. L. Rosenthal and C. W. Tobias say

measurement of blood electrical resistance is useful in studying variations in coagulation and clot retraction (*J. Lab. Clin. Med.*, 33/1110/48). P. J. Crittendon and others report decreased temperature, gastric acid, urine, and muscle contractility in biotin deficiency (*Arch. Internat. Pharmaco. Therap.*, 77/29/48). W. D. Armstrong and others find radiactive carbon generally distributed in all parts of body (*Proc. Soc. Exp. Biol. Med.*, 68/233/48). L. Horlick and others find cholesterol is removed from blood rapidly when content is high, slowly when low (*ibid.*, p. 245). G. A. Paff reports that more bone is formed and more calcium deposited at low than at high pH (*ibid.*, p. 288). Our D. Duncan shows that nerve constriction causes myelin loss and slows growth (*J. Neuropath. Exp. Neurol.*, 7/261/48).

3. FURTHERMORE: Our J. A. Scott continues standardization of filarial experimental infections (*Am. J. Trop. Med.*, 28/481/48). Our A. Packchanian shows triatoma are no hazard in possible spread of African sleeping sickness in America (*ibid.*, p. 541). A. Sole develops concept of "anergene," a substance inhibiting allergic reactions (*Wien Klin. Wchnschr.*, 60/493/Aug. 6/48). O. K. Behrens and others biosynthesize many new penicillins of high potency (*J. Biol. Chem.*, 175/793/48). Note symposium on urethane in leukemia (*Blood*, 3885–917/48). A. V. Majoska challenges medical detectives with new syndrome of sudden death in Filipino men (*Hawaii Med. J.*, 7/469/48). Note appropriate symposium on centennial of *Virchow's Archives* by R. Rossle, P. Diepgen, and F. Munk (*Virchow's Arch. Path. Anat.*, 315/1–45/48). R. F. Fleming and O. Peczenik find normal male urine growth inhibitors reduce tumor alkaline phosphatase. (*Nature*, 162/338/Aug. 28/48). J. F. Burgess recommends Vitamin E in collagenoses (*Lancet*, 2/215/Aug. 7/48).

NOVEMBER, 1948

CLINICAL: K. Setala and P. Ermala show radiation sickness symptoms correlate with change in blood lipids detectable by chylomicrography (*Ann. Clin. Gyn. Fenniae*, 37/Suppl. 1/48, 60 pp., Helsinki). K. Z. Morgan discusses protection against radiation hazards and maximum allowable exposure values (*J. Indust. Hyg. Tox.*, 30/286/48). H. Koehler and O. Koenig discuss influence of lactoflavin on water and salt metabolism in dermatoses (*Arch. Dermat. Syph.*, 186/669/48). G. Lu suggest sparteine is superior to quinidine for clinical use in cardiac irregularities (*Arch. Internat. Pharmacody*, 76/367/48). W. E. Ricketts notes serious prognosis in bartonella anemia (*Blood*, 3/1025/48). L. Aldana and others find streptomycin effective in bartonellosis or Carrion's disease and Oroya fever (*Arch. Peru Path. Clin.*, 2/323/48). L. C. Brumpt and B. J. Gujar recommend treating polycythemia with artificial hookworm infection (ancylostomotherapy) as safe and effective (*Indian Med. Gaz.*, 83/166/48). L. P. Rowland and F. A. Mettler conclude that coronal suture is not reliable guide to cerebral anatomy in prefrontal lobotomy (*J. Comp. Neurol.*, 89/21/48). C. P. McCord salutes the harried train dispatcher (*Industr. Med.*,

17/377/48). C. L. Conley and others describe a circulating anticoagulant as a cause of hemorrhagic disease (*Bull. Johns Hopkins Hosp.*, 83/288/48). D. C. Sinclair and others discuss referred pain and associated phenomena (*Brain*, 71/184/48). I. L. Marner finds thymol turbidity test useful in differentiating hepatitis from obstructive jaundice (*Acta. Med. Scand.*, 131/180/48). J. Gillman and others report high incidence of congenital malformations in rats as result of abnormal particles (as rubella virus or protein-binding dyes as trypan blue) in blood (*S. Afr. J. Med. Sci.*, 13/47/48). M. R. Castex and others recommend succinic acid as respiratory stimulant in depression by barbitals (*Prensa Med. Argent.*, 35/1327/July 9/48). W. Schonfeld offers interesting historical account of P. Ricord (1800–1889) and syphilis (*Arch. Dermat. Syph.*, 186/459/48). Our R. Gregory discusses basic problems of hypertension (*New Orleans Med. Surg. J.*, 101/141/48). R. Lium and S. Maddock say acute pancreatitis results from ducal obstruction in an actively secreting pancreas (*Surg.*, 24/593/48). Clear, succinct, stimulating and helpful is J. H. Burn's *Background of Therapeutics*. Get it! (Oxford Univ. Press, N.Y., '48, 367 pp., $6). Two useful monographs are D. G. Anderson and C. S. Keefer's *Therapeutic Value of Penicillin* and C. S. Keefer and W. L. Hewitt's *Therapeutic Value of Streptomycin* (Edwards, Ann Arbor, Mich., '48, 314 pp., 297 pp., $7 each).

PHYSIOLOGICAL: E. R. Norris and J. J. Majnarich find *in vitro* marrow growth accelerated by factor in normal blood sera but inhibited by factor in sera of pernicious anemia, leukemia and cancer—also that xanthopterin and normal sera inhibit neoplastic growth *in vitro* (*Am. J. Physiol.*, 153/483–498/48). E. De Robertis and F. O. Schmitt report nerve degeneration related to disintegration of neurotubules (*J. Cell Comp. Physiol.*, 32/45/48). H. Fischer and R. Frohlicher show restorative action of adenosinetriphosphate ATP on fibrillescent heart (*Helv. Physiol. Pharmacol. Acta.*, 6/196/48). W. Wilbrandt and H. Koller show that calcium heart effect depends quantitatively on concentration at the membrane (*ibid.*, p. 208). B. Uvnas in one of many reports finds a pepsigogue principle in pyloric and duodernal mucosa extracts. (*Acta. Physiol. Scand.*, 15/438/48). H. W. Bischoff proposes a hemodynamogram for analyzing fetal, healthy and abnormal blood circulations (*J. Pediatrics*, 33/401/48). P. L. Mollison, A. R. Mourant, and R. R. Race offer *Rh Blood Groups and Their Clinical Effects* (Med. Res. Counc. Memo. 19, '48, HMSO, London, 1s6d). J. E. LuValle and D. R. Goddard survey mechanism of enzymatic oxidations and reductions, proposing ternary complexes with semi-quinone formation (*Quart. Rev. Biol.*, 23/197/48). D. G. Frost finds little relation between nutritional deficiency and our graying hair (*Physiol. Rev.*, 28/368/48).

PHARMACOLOGICAL: S. Rowlands and others, using radiactive S penicillin confirm rapid urinary excretion and slow intestinal absorption. (*Lancet*, 2/493/Sept. 25/48). E. Friedmann and others report antimitotic action of maleic acid related to conjugated double bonds as in quinones, colchicines and stilbestrols (*Brit. J. Pharmacol.*, 3/263/48). C. A. Pfeiffer and E. Allen

found it impossible to cause cancer in rhesus monkeys by carcinogenic hydrocarbons and estrogens (*Cancer Res.*, 8/97/48). C. A. Winter reports that antihistaminic drugs potentiate barbital sedation (*J. Pharmacol.*, 94/7 48). S. W. Landon and others compare antihistamine substances (*Bull. Johns Hopkins Hosp.*, 83/330–365/48). E. B. Schwenbach and E. M. Greenspan review pharmacology of aromatic diamidines (*Medicine*, 27/ 327/48). G. S. Eadie notes that methadon inhibits brain cholinesterase (*J. Pharmacol.*, 94/19/48). Note well the important review by K. B. Augustinsson on Cholinesterases (*Acta. Physiol. Scand.*, 15, Suppl. 52, '48, 182 pp.).

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